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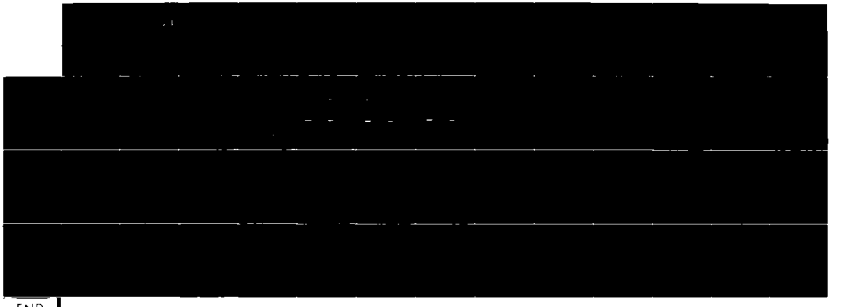

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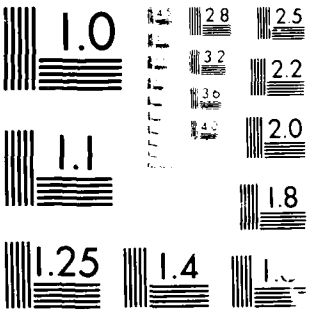
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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

U.S. military combat personnel will not always be able to maintain normal 24 hr schedules of work and rest. The work in this contract was directed toward modeling such non-24 hr environments in order to learn their consequences on physiological function, ability to perform a task, and susceptibility to succumbing to a disease process. To do this, we divided the work into 2 parts. The first of these used rodent models to study susceptibility to succumbing to disease. The first of these used a genetic model for heart disease -- the cardiomyopathic hamster. The second used activity-stress. In both models, we found that living in constant light, an extreme example of a non-24 hr environment, had a protective effect. Since one of the reasons for this effect related to a photoperiodic effect, we studied biological rhythms in hamsters living in long and short days and found that in contrast to those animals living in long days, those living in short days lost their circadian hormone rhythms.

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19. ABSTRACT (cont'd)

The second major thrust was to develop a non-human primate model on which to study the effects of non-24 hr environments. The major data reported here come from our original model which employed the chronically chair-adapted monkey, but recently we have perfected the telemetry system required to do these studies in free-ranging monkeys and are currently collecting data in this improved model. We found that the model we developed was an excellent one in that it provided data identical to those reported in man but at only a fraction of the cost. The cost factor let us continue our studies longer than is ever done with humans and thus we noted some finding never previously reported in humans -- i.e., following simulated jet lag, the animal has a second period of performance impairment that occurs 10-14 days after the phase shift -- at a time when other circadian rhythms are well entrained. Using this model, we learned that living in timeless environments has behavioral consequences and that demanding that a monkey work for its food could entrain circadian rhythms while simply allowing the monkey restricted access to food did not.

Finally, we showed that the monkey in these conditions could be used in studies of constant operations as a monkey will work continuously at high efficiency for many hours. We believe the model we have developed has applications relevant to military needs which go beyond the area of chronobiology. These include drug effects in conop states, the effect of shift work on different tasks and time of day effects on performance with and without the superimposition of performance-altering drugs.

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THE PATHOPHYSIOLOGY OF CIRCADIAN AND ULTRADIAN RHYTHM DISTURBANCES ON BEHAVIORAL AND VISCERAL FUNCTIONS, STRESS RESPONSE, AND DISEASE SUSCEPTIBILITY

Final Report

Benjamin H. Natelson, M.D.

Walter N. Tapp, Ph.D.

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FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 86-23, Revised 1985).

BACKGROUND AND AIMS

Throughout military history, words such as "dawn," "dusk," "day" and "night" have had important strategic significance to military planners. But this is no longer the case. Since battles may occur at any time and may rapidly shift back and forth across time zones, day-night boundaries have become less important to military planners. The application of modern technology to warfare further blurs the field commander's dependence on daylight: Long range weaponry and computer control suggest that control centers will replace the bunker as the theatre of war. In such centers, "LIGHTS OFF" will be one command which is never heard. Obviously, such a major change in the "rules" of modern warfare carries with it a whole host of unknown consequences.

The goal of the Primate Neuro-behavioral Unit under this contract was to model situations with altered environmental time cues and/or work-rest schedules in order to provide data to help military planners understand some of the consequences of these conditions. There have been a number of specific areas we have addressed during the course of this contract. A major one of these was to ask the question of what the health consequences would be of living in environments with altered time cues or disrupted work-rest schedules. Another question was directed to an examination of physiological changes that occur during life in such conditions. Another major goal was to develop a model which could be easily applied to questions conerning life in such conditions.

To get answers to these questions, we divided our efforts along 2 major avenues. To understand the health consequences of life in environments with altered or absent time cues, we used rodent models. Our design strategy for this part of the work was to place animals with either spontaneous disease or with some predisposition to disease in environments with non-24 hr light-dark schedules and observe the influence of these environmental differences on the course of disease. As subjects for this work, we used a strain of hamsters (CMH) which inherit a form of heart disease, and we used activity-stress as a disease enhancing procedure to sensitize otherwise normal rats and degus. To learn how life in these situations alters physiology and performance, we have used the chronically instrumented rhesus monkey.

RESUME OF WORK ACCOMPLISHED

Rodent Research Overview. In deciding which non-24 hr environment to use, we decided to use one totally devoid of time cues -- i.e., constant light. Such conditions had been thought to be particularly stressful as the animal's biological clock has no information with which to be entrained. Our first experiment was to assess the effect of constant light on CMH. That experiment showed that constant light was protective (Tapp & Natelson, Lancet i:239-40, 1986). Hamsters living in constant light lived as much as 25% longer. Moreover, we learned that the progress of their congestive failure was slowed by the light treatment.

The results of this experiment were startling. Instead of sensitizing the hamster to succumb quicker to its disease because of the "stress" of living in an environment devoid of time cues, the animal was actually protected. Our immediate task was to begin trying to understand what about constant light was protective. One possibility immediately came to mind. Our control group was hamsters living 12 hr light and 12 hr dark. Studies have been done using healthy hamsters which indicate that these animals treat a 12 hr day as a "short day." The hamster is very sensitive to light duration in a 24 hr day. On short days (i.e., short photoperiods), the hamster becomes reproductively inactive (due to testicular regression) while it returns to reproductive activity (with a return to normal testicular function) in the long photoperiods that reflect lighting conditions of spring and summer.

Thus, one possible explanation for the life extending effects of our first experiment was that a longer photoperiod (i.e., as would be the case with constant light) was healthful. An obvious first step was to determine whether CMHs responded to photoperiod in the same way as the healthy hamster. We did an experiment to show that they do (see publication #7 in list of publications supported by this contract; numbers in parentheses that follow refer to other such publications in that list). Hamsters living in 12 hr of light per 24 hr day treat the 12 hr as a short photoperiod and undergo testicular regression with its accompanying decrease in plasma testosterone. Animals living in constant light treat that regimen as a long day.

So this experiment made it clear that differences in photoperiodism could have been responsible for the life extending effects of constant light. However, several other effects of light also could have been operative. In the constant light condition, hamsters are getting a large "dose" of light and also are living in a timeless environment where their biological clock free runs. To assess these possibilities, we began an experiment designed to indicate the lighting mechanism responsible for the prolonged life effect. Unfortunately, with the cessation of funding, we were unable to finish that long-term study.

However, we did do another experiment designed to explore some of the chronophysiological consequences of living in short of long days (6). Ottenweller et al. showed that healthy hamsters living on long days had normal rhythms of adrenal, thyroid and testicular hormones but hamsters living on short days did not. Morover, assessment of integrity of the reproductive system (i.e., weight of testes, seminal vesicles and epididymides) revealed hypofunction in the short day group. Thus this work recapitulates our assessment of gonadal function in the CM hamsters. It should be noted however, that several studies have been done which indicate that gonadectomy extends life in otherwise healthy populations (e.g., J. Gerontol., 20:96, 1965 and 24:395, 1969). If this effect of reduced gonadal function could have been extended from the normal aging process to the situation when disease ends life, one might have expected the short day group -- not the long day group -- to have been protected in our original studies. Since this was not the case, it suggests that the endocrine consequences of living in short or long days were not responsible for the life extension found. Of course, an alternative explanation is that it is not healthy to live devoid of endocrine circadian rhythms. Unfortunately, this remaining issue will have to stay unanswered for the time being.

Another area in which we began to work was to try to find rodent-like animals that were thought to be day-active rather than night-active. The

rationale for doing this was that we were doing all our mortality work in the night-active rodents and the majority of our physiology in the day-active monkeys (see below), and we thought it important to study mortality in a day-active species to begin bridging from the rodent to the primate. After evaluating the mammology literature, we decided to study the degu, a Latin-American rodent that was said to be day-active. After establishing a breeding colony of these animals, we sent breeding pairs to WRAIR where a separate breeding colony was established for use by WRAIR scientists. The first issue we wanted to establish was whether in fact these animals were day- or night-active. We learned that this was a complicated question. For example, when rat cage activity is studied, it is seen that rats start moving about late in the daytime and then continue activity throughout the night. Since the majority of their activity occurs at night, they are considered nocturnal. The degus began their activity uniformly during the day but continued active throughout half the night. And in fact, they had about as much activity in the day as in the night. Thus, based on the time of activity onset, they were diurnal, but in terms of total activity, they were neither diurnal nor nocturnal. The other thing we did with these animals was to run them in the activity-stress paradigm in two lighting conditions -- constant light and L:D 12:12. As we had found in our initial work on lab rats, degus living in constant light did not succumb to activity-stress while those living in LD did. Importantly, there was no significant difference between distance run in the 2 experimental groups. Thus, this experiment is important in making the point that the disease-resisting properties of life in constant light hold for other processes besides innate heart disease and hold for other species too.

Monkey Research Overview. A major goal of this project was to develop a non-human primate model that could be applicable in an assortment of different situations in which performance and biological function needed to be studied over time. We have achieved this goal. The enclosed paper (9) details the method and its application in jet lag. The paper is seminal in that it is the first time in animal chronobiology that performance measures have been taken in association with chronobiological ones. Although this is routinely done in humans, human studies have the drawback of being extremely expensive, and frequently, the performance measure is not one which the individual is performing asymptotically. Thus, effects on learning are sometimes confounded with effects on performance. This is not the case with the monkey model. The animal works on its task under time constraints which we control, and since the task remains the same, small differences in performance become highly significant. This was seen in the paper enclosed. Following a 6 hr phase advance to simulate jet lag, the expected performance decrement was found at the time of physiological rephasal. Importantly, however, a second significant and striking performance decrement was found 10 days later. This means that even after chronobiological function has returned to normal, a performance decrement occurs. Thus the decrement cannot be a function of the transient internal desynchronization that occurs following the phase shift. Some other explanation must be operative. The observation itself is important to operational planners because it indicates that a performance decrement will occur up to 2 weeks after a transmeridional flight.

Much of the data used in preparing this report came from our original model which used the monkey living chronically in the primate chair. But a major goal of this contract was to develop the technology to move the model to the free ranging macaque. We can report our success in achieving this. Dr. Stan Reisman, our electrical engineering colleague, developed the circuity

to allow the study of 6 different telemetered temperature signals from 6 animals living within the same room. Our review of other off-the shelf technology did not find anything with this capability which could be used with large mammals. Because of this development, during the latter part of the contract, we moved all 6 of our monkeys from chairs to large cages which allowed them unrestricted motion. Cages were built on a tilt apparatus to allow activity counts and off-the-shelf temperature telemetry units were implanted in each animal. VAX-based software was developed to support the enormous amount of data generated. A major point for future consideration is that this model could be used for other military-related applications besides chronobiological studies. Some of these other applications should become clear by the end of this report.

The next issue we addressed was what controlled entrainment of the temperature and activity rhythms. It was originally thought that restricted access to food could entrain these rhythms but recently it has become clear food restriction only masks these rhythms. We gave three monkeys free access to food for 8 hr a day and another 3 monkeys access to task-contingent food. We found that the free feeding monkeys either free-ran or showed masking but the task-contingent feeders showed true entrainment of their temperature and activity rhythms (4). These data indicate that work load can be used to aid in entraining biological rhythms. This means that imposing tasks on an individual could be used to help speed re-entrainment after jet lag. This finding could have significance for operational planners.

Following our having done these studies, monkeys were released into constant light. The first thing we found (2,3) was that although the monkeys worked the same 8 hr shift, doing so in constant light produced a performance decrement. While the deficits seen were somewhat less severe than those seen following jet lag, they were much longer lasting, persisting for over 100 days without sign of recovery. Importantly, during this prolonged period of impaired performance, temperature and activity rhythms showed stable performance. Thus constant light represents a lighting condition that produces significant performance deficits despite the fact that internal desynchronization does not exist. In combination with our earlier observation, this means that internal desynchronization cannot be used as a mechanism to explain performance deficits that occur after phase shifts. Finally, an inference can be drawn from this experiment and that is that non-24 hr LD schedules can impact in important ways of performance. Evidently, confirming this inference will require further experiments in which monkeys lived in other non-24 hr schedules besides constant light.

Following this step, the monkeys were allowed around-the-clock access to either the task (and thence to their food) or to free food. This manipulation again had effects on levels of performance (5). The decrease in vigilance performance noted probably occurred simply because the monkey had so many trials that it could work for food any time it wanted; thus the existence of satiation would decrease motivation. But motivational explanations cannot be used to explain the 16.5% improvement in discrimination performance that was found when this condition was compared to the one in which the task was available only 8 hr per day. Another important thing that was seen here was that the phase relations between vigilance and discrimination performance changed when the monkeys were moved from an 8 hr work shift to around-the-clock access to food. In the former condition, vigilance peaked in mid-shift while discrimination peaked early in the shift, and, as might be inferred, there was no significant relation between performance on the 2 tasks when data

were explored along the time dimension. Parenthetically, it should be noted that these phase relations are the same as those reported in humans performing simple repetitive tasks and more cognitively demanding tasks, respectively. However, when the task was available around-the-clock, the phase relations of the 2 tasks approached one another, and a highly significant correlation between the two performances was seen. We believe these data suggest that changes in the internal phase relations of rhythms during free-runs may improve performance on more complex tasks.

Our next major observation had to do with anomalies in circadian rhythms that were seen in these conditions (8). Free feeding monkeys showed a rather consistent anomaly which was variable in duration but lasted as long as 97 days. Temperature would free run at a circadian frequency (usually just under 24 hr), but food intake and activity would lose its circadian rhythm and instead show just an ultradian rhythm. Explanations for this can be found in the enclosed paper (8). But it is important to highlight that this anomaly was seen only in the free feeding group. Equally interesting was another anomaly which was seen only in the task contingently fed monkeys. Two of 9 monkeys showed brief periods of internal desynchronization where the circadian rhythms of performance and temperature were significantly different (see enclosed paper for conclusions).

The final thing we explored before the termination of the contract was to determine whether the model could be used for the study of constant operations. We understand that this is an area toward which Army planners are directing a considerable amount of energy. To test this, we changed the schedule of reinforcement for one monkey. Whereas in the past, the monkey was assured of getting a food pellet after every successful trial during its 8 hr work shift (i.e., on the average, every 2.4 min), we made only one of three successful trials pay off. And we did this after omitting one day's shift so that the monkey was hungry and thus would be motivated to work constantly. We programmed the monkey to work for the next 72 hr. The animal worked without cease for the first 24 hr; during the second 24 hr, the animal worked for 22 hr but interruptions were for never longer than 20 min. In the final 24 hr, the monkey took more time outs and these ranged from 40-60 min. Of the 1800 consecutive trials, the monkey missed only 8.6%. An analysis of successful trials showed that the median latency to respond averaged 46% longer during the period of conops than during the period when the monkey worked the 8 hr shift (1.65 sec and 1.13 sec respectively). Thus as expected vigilance was affected by conops. Similarly, latency to make the discrimination was impaired also (compare medians of 2.3 sec during conops with 1.5 sec during shift work). We believe these are important pilot data because they indicate the monkey can be used in studies of constant operations in situations where humans cannot be used -- e.g., to test the effects of drugs which cannot currently be used on people.

In summary, we have developed a model which permits continuous assessment of activity, temperature and feeding behavior in free-ranging, non-human primates which are performing a task either in a clearly defined shift or around-the-clock. We have applied this model to chronobiological questions but it should be clear that the model could be applied to inquiries concerning time of day effects (with or without drugs) on performance and physiology as well.

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PUBLICATION 1

CIRCADIAN FACTORS IN MONKEY PERFORMANCE: TASK DIFFERENCES AND DEFICITS AFTER PHASE SHIFTS. B.H. Natelson, D. Creighton, W.N. Tapp. VA Medical Center & New Jersey Medical School, East Orange, NJ 07019.

We have developed a task where rhesus monkey performance exhibits important features of circadian influences on human performance. The task is a chained vigilance-discrimination task. A white cue light comes on to signal the onset of a vigilance trial. The monkey has 10 seconds to press a lever to successfully complete the trial. Immediately following a successful vigilance response, a red or green light comes on and the monkey has 10 seconds to press the associated lever on the right or left. A food pellet is delivered after successful completion of this sequence. Monkeys were trained to asymptotic performance in LD 12:12. Temperature and activity rhythms were also monitored continuously throughout the experiment. During stable entrainment discrimination performance peaked an average of 3.88 hours before vigilance performance, corresponding to task differences in humans where performance on "more cognitive" tasks peaks before performance on simple repetitive tasks. Phase shifts (± 6h) produced performance deficits in both discrimination ($p < .02$) and vigilance ($p < .01$). Discrimination performance recovered significantly faster than vigilance performance ($p < .01$), corresponding to task differences in human recovery from jet-lag following circadian phase shifts. A second, shorter, milder deficit was found 10-14 days after the phase shift.

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CIRCADIAN FACTORS IN MONKEY PERFORMANCE: EFFECTS OF TASK, PHASE SHIFTS AND CONSTANT LIGHT. W. N. Tapp*, B. H. Natelson, D. Creighton*. (SPON: S.D. Cook). Primate Neurobehavioral Unit, VA Medical Center & New Jersey Medical School, East Orange, NJ. 07019.

We have developed a task where rhesus monkey performance exhibits important features of circadian influences on human performance. Monkey performance on this chained vigilance-discrimination task exhibits task dependent differences in the time of peak performance (discrimination peaks several hours before vigilance, $p<.01$) and in the time required to recover from performance deficits following a 6 hr phase shift (discrimination recovers significantly faster than vigilance, $p<0.01$). These task differences correspond to differences in humans where performance on "more cognitive" tasks peaks earlier during the day and recovers more rapidly after a phase shift than performance on simple repetitive tasks. Similarly, phase shifts produced the same sort of transient internal desynchronization in monkeys that they do in people, with activity resynchronizing significantly faster than temperature.

After characterizing performance in LD 12:12, we released the monkeys into constant light (LL). The task was available in LL from 0900 to 1700. Monkeys showed significant performance deficits in LL. Average vigilance performance was 15.1% worse in LL than in LD 12:12 ($p<.01$). Average discrimination performance was 14.6% worse in LL than in LD 12:12 ($p<.01$). While these deficits were not as severe as those associated with phase shifts, they were much longer lasting, persisting for over 100 days without sign of recovery. Performance deficits in LL were not due to internal desynchronization. During the period studied, the monkeys' circadian temperature and activity rhythms maintained stable periods and phase relationships. Indeed, both rhythms were synchronized to the time of the task with 24 hr periods and positive phase angles with respect to the task. Thus, LL represents a light schedule that produced significant performance deficits despite the fact that the monkeys were internally synchronized.

The pattern of behavioral change and physiological rephasal shown by rhesus monkeys after phase shift closely resembles that seen in humans. Thus, this model provides a unique opportunity to study performance and physiological function after chronobiological manipulations that would be too expensive or impossible to study in humans.

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Symposium

Behavioral Medicine: Theory and Treatment

Moderator: B. T. ENGEL

W. E. WHITEHEAD (NIA, Baltimore, Maryland)—The psychophysiology of irritable bowel syndrome

H. SHABSIN (NIA, Baltimore, Maryland)—Classical conditioning in the treatment of irritable bowel syndrome

B. ROLLS, M. HETHERINGTON (Johns Hopkins School of Medicine)—Changing hedonic responses to foods

M. HETHERINGTON, B. ROLLS (Johns Hopkins School of Medicine)—Hunger and satiety in eating disorders

G. BIGELOW (Francis Scott Key Medical Center/ Johns Hopkins School of Medicine)—Relapse in addictive disorders. Relapse prevention is increasingly recognized as the critical issue in the treatment of addictive disorders. A motivational conceptualization of the determinants of relapse is proposed. Abused substances—drugs, alcohol, tobacco—function as biologic reinforcers maintaining the substance self-administration behavior of abusers. Relapse can be reduced by scheduling countervailing behavioral contingencies that either provide an incentive for abstinence or promote avoidance of substance use. Data from both laboratory and clinical studies of human substance use illustrate the efficacy of this motivational approach to understanding and influencing relapse to addictive disorders.

C. RAND (USA)—In hospital smoking relapse following an MI: an unexpected intervention

SESSION III

Moderator: B. H. NATELSON

B. H. NATELSON, D. CREIGHTON, W. N. TAPP (VA Medical Center and New Jersey Medical School, East Orange, New Jersey)—The effect of performance demands and environments without time cues on biological rhythms in rhesus monkeys. Ultradian rhythms, those with a frequency faster than one cycle per 20 hours, have been described as wobbling and broad-based. But prior evaluations of such rhythms have used either short data sets or discontinuous types of data, such as meals. We have studied core temperature, activity, and feeding in six chair-adapted rhesus monkeys living in LD 12:12, three of these earned their food by working 8 hours/ day on a vigilance-choice task, while the others had free access to food. Five blocks of data (five days each) were analyzed. Following appropriate preprocessing and fourier transformation, averaged spectra revealed identical patterns for all six monkeys; peaks were seen at about 24 hours, 3 hours, between 80 and 90 minutes, and at about 40 minutes. When monkeys were released into constant light, somewhat broader peaks were seen but the identical pattern still remained. These data indicate that stable ultradian rhythms exist, and remain despite performance demands or removal of temporal cues. The fact that their robustness has not been appreciated before may relate to the earlier interest in circadian chronobiology or to the fact that inadequate amounts of data had been evaluated.

F. J. McGUIGAN, A. DOLLINS (United States International University)—Does the speech musculature really generate a phonetic code? Evidence was reviewed from previous studies that confirmed the hypothesis that there is a discriminative relationship between the phonemic system and speech muscle region. More specifically, during the silent processing of verbal labial information (*e.g.*, bob, mom) the lips are covertly activated, as indicated by relatively heightened electromyographic activity. Conversely, the tongue is relatively active when one is processing lingual-alveolar information (*e.g.*, dad, tot). Recent evidence presented in preliminary fashion confirms further the discriminative relation hypothesis known as the McGuigan-Winstead Effect. We therefore have additional reason to believe that the speech musculature generates a phonetic code that is transmitted to and from the linguistic regions during semantic processing.

W. HORSLEY GANTT MEMORIAL LECTURE

Moderator: P. R. McHUGH

J. W. MASON (Yale University School of Medicine)—Some historical and conceptual issues in psychoneuroendocrinology

PAVLOVIAN SOCIETY BANQUET

President: J. V. BRADY—Extemporaneous remarks

USSR CENTRAL TELEVISION AND THE SOVIET ACADEMY OF SCIENCES—(Film) Pavlov: the conditioned reflex

292.8 ENTRAINMENT OF MONKEY CIRCADIAN RHYTHMS: EFFECTIVENESS OF FOOD RESTRICTION AND TASK DEMANDS. W.N. Tapp*, D. Creighton*, T.A. Frissel and B.H. Natelson (SPON:F.S. Knox). VA Medical Center and New Jersey Medical School, East Orange, NJ 07018.

Six adult, male rhesus monkeys were individually housed in chambers designed to enable us to control their environment. Three monkeys were required to perform a chained vigilance-discrimination task to obtain their food. Trials occurred on the average of every 2.4 min in the task. The other three monkeys merely had to press a key to obtain food. Activity, feeding and temperature rhythms were monitored in all monkeys.

In the first phase of the experiment (Constant Task/Free Feed), the task ran around the clock and food was available ad lib for non-task monkeys. During this phase, all monkeys exhibited normal free-runs with activity, temperature, and performance or feeding (in the non-task monkeys) showing the same period and stable phase relationships within a given monkey. In the second phase of the experiment, task and food availability were restricted to 8 hr/day. Both temperature and activity synchronized well to the time of task availability in monkeys that were working for their food. Synchronization was much poorer for the non-task monkeys, and one monkey even maintained a clear free-running component throughout the restricted feeding part of the experiment. Finally, monkeys were released to free-run in Constant Task/Free Feed. Monkeys were released on days when the onset of subjective day predicted from their initial free-run was about 12 hr out of phase with the onset of restricted task or feeding. Activity, temperature and performance all began to free-run smoothly from the phase of restricted task, indicating that the periodic task demands/restricted food combination truly entrained these rhythms. In contrast, non-task monkeys either consolidated their rhythms in the free-running component that was visible during restricted feeding or shifted toward the phase predicted by their initial free-run, indicating that restricted feeding alone did not successfully entrain activity, temperature or feeding.

We cannot tell whether task demands alone would entrain the rhythm or whether the task/restricted feeding combination is necessary. However, the results indicate that periodic cognitive or work demands can play a role in entrainment of primates.

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FREE-RUNNING CIRCADIAN PERFORMANCE RHYTHMS IN MONKEYS. W.N. Tapp and B.H. Natelson. Primate Neurobehavioral Unit, VA Medical Center and Department of Neurosciences, New Jersey Medical School, East Orange, NJ 07019.

We have developed a monkey performance model for examining the effects of time-of-day, circadian rhythms, and work-rest schedules on the efficiency of the performance of learned tasks. In this vigilance-discrimination model, rhesus monkeys must perform a two-component task to obtain food. The first component is a vigilance task, where the monkey must detect the onset of a trial and respond with a lever press within a time limit. Success on the vigilance task initiates a discrimination task where the monkey must press either a right or left lever, depending upon whether a red or green cue light comes on. Choices and response latencies are recorded on computer. The monkey's temperature and activity rhythms are recorded every 10 min around the clock. In previous work, we found strong time of day effects that differed in their peak time for the two tasks, and we found that a 6 hr phase advance produced a jet-lag-like syndrome that included significant performance deficits. However, the monkeys were working 8 hr sessions in entrained conditions in those studies. The current study was designed to examine the circadian characterstics of performance by determining whether (1) periodic performance variations are found in constant conditions, (2) the effects of free-run on overall performance, and (3) its effects on task-dependent performance patterns.

We studied the performance of three adult, male rhesus monkeys in constant light with trials presented around the clock on a variable interval schedule averaging one trial every 2.4 min. All 3 monkeys exhibited free runs as long as they were maintained in these conditions (up to 180 days). The monkeys exhibited free-running performance periods of 23.4 hr, 23.7 hr and 24.6 hr. Performance differed significantly from the levels seen in the 8 hr task in entrained conditions. Vigilance performance declined by an average of 30.7% $\pm$ 8.1 SEM ($p < 0.01$). The decline in vigilance may be due to the fact that monkeys were able to eat significantly more food in the continuous task than in the 8 hr task ($p < 0.01$). However, discrimination performance improved an average of 16.5% $\pm$ 1.2 SEM ($p < 0.01$) in free-running conditions. It seems unlikely that increased performance in constant conditions is due to decreased food motivation. Instead, the data suggest that changes in the internal phase relationships of rhythms during free-run may improve performance on more complex tasks.

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Adrenal, thyroid, and testicular hormone rhythms in male golden hamsters on long and short days

JOHN E. OTTENWELLER, WALTER N. TAPP,
DAVID L. PITMAN, AND BENJAMIN H. NATELSON
Primate-Neurobehavioral Unit, Veterans Administration Medical Center, East Orange 07019; and Department of Neurosciences, New Jersey Medical School, Newark, New Jersey 07013

OTTENWELLER, JOHN E., WALTER N. TAPP, DAVID L. PITMAN, AND BENJAMIN H. NATELSON. *Adrenal, thyroid, and testicular hormone rhythms in male golden hamsters on long and short days.* Am. J. Physiol. 253 (Regulatory Integrative Comp. Physiol. 22): R321-R328, 1987.—Plasma concentrations of adrenal, thyroid, and testicular hormones were measured at 4-h intervals around the clock in male hamsters on long (14:10-h light-dark cycle) and short (10:14-h light-dark cycle) days. Plasma corticosterone, cortisol, thyroxine (T_4), triiodothyronine (T_3), and testosterone rhythms were present on long days. The only one of these hormones to have a significant rhythm on short days was cortisol, but even its amplitude was suppressed compared with the cortisol rhythm on long days. Short days also lowered mean plasma levels of cortisol, T_4, T_3, and testosterone. Finally, short days raised the ratio of corticosterone to cortisol and lowered the ratio of T_4 to T_3. Both ratios had significant rhythms on long days but not on short days. Because of the many interactions among adrenal, thyroid, and testicular hormone axes, it is unclear whether the primary effect of short days is on one of these endocrine systems or on another factor that has separate effects on each of the hormone rhythms that was measured. Nonetheless, it is clear that a major effect of short day lengths in hamsters is to suppress hormone rhythms. Explanations of photoperiodic effects that depend on endocrine mediation should take this into account.

circadian rhythms; corticosterone; cortisol; thyroxine; triiodothyronine; testosterone; photoperiodism

GOLDEN HAMSTERS have long been known to be photoperiodic, and their measurement of day length appears to be explained by a model in which light acts as both an entrainer of a photoinducible phase and as a stimulator during this photoinducible phase (10). Meier (18) has proposed an extension of this model for vertebrates in which the two actions of light are reflected in the phase relationships between hormone rhythms, which may have a role in mediating photoperiodic effects. His laboratory has recently shown that the phase relationships between cortisol and insulin rhythms may vary depending on season and day length (9). In addition, others have measured plasma glucocorticoid (2) and thyroid hormone (34) rhythms in hamsters but only on long photoperiods. The present studies extended this research by measuring the effect of day length on the daily patterns of plasma corticosterone, cortisol, thyroxine (T_4), triiodothyronine (T_3), and testosterone concentrations in hamsters maintained on long and short days.

Plasma hormone levels in these systems have been shown to be rhythmic in most vertebrates including humans. The earliest reports of hormone rhythms involved glucocorticoid rhythms in humans and rats. We have recently shown that both corticosterone and cortisol are present in significant amounts in hamster plasma (24), and Albers et al. (2) have reported daily rhythms of both hormones in hamsters on long days. Testosterone rhythms have also been examined in both humans (26) and rats (39), but we are not aware of any reports of testosterone rhythms in hamsters. Finally, although the presence of plasma T_4 and T_3 rhythms had been disputed, most recent studies have reported both T_4 and T_3 rhythms in humans (4) and rats (22). Vriend (34) has recently shown that plasma T_4 and T_3 have daily rhythms in hamsters on a long photoperiod.

Although there have been relatively few reports of hormone rhythms in hamsters, more studies have examined how manipulating mean hormone levels can alter rhythms in behavior or other hormones. This is important for understanding the data from this study because we found several effects of day length on mean hormone levels. In this regard, one of us has previously reported that the amplitudes of both plasma corticosterone and prolactin rhythms in rats were dependent on plasma levels of thyroid hormones (21). In addition, changing either thyroid or testicular hormone levels can affect various aspects of free-running and entrained behavioral rhythms (1, 19), and there is some evidence that glucocorticoids might affect activity rhythms (14). Thus some effects of day length on hormone rhythms in the current study may be due to interactions among the hormone systems such that changes in the overall levels of one hormone may affect the rhythms of other hormones.

METHODS

Male hamsters (CHF 148), 8-12 mo of age, were obtained from Canadian Hybrid Farms (Halls Harbor, Nova Scotia, Canada) where they had been maintained on a 18:6-h light-dark (LD) schedule from birth. These hamsters were placed on a 12:12-h LD schedule for 3 mo in our animal quarters. At 11-15 mo of age the hamsters were transferred to either a 14:10-h LD schedule with lights on from 0730 to 2130 (long days) or a 10:14-h LD schedule with lights on from 1030 to 2030 (short days). After 15 wk on these photoperiods, hamsters on both photoperiods were killed at 4-h intervals around the clock

beginning at 0800. Hamsters were killed by decapitation, and trunk blood was collected into heparinized centrifuge tubes. The blood was centrifuged, the plasma was collected, and aliquots of plasma were stored frozen at −40°C until hormone assays were performed. After death the adrenals, testes, seminal vesicles, and epididymes were removed and weighed.

Radioimmunoassays (RIA) were performed for plasma corticosterone and cortisol (24), T_4 and T_3 (25), and plasma testosterone. Plasma corticosterone was assayed using the (B3-163) antibody from Endocrine Sciences (Tarzana, CA), tritiated corticosterone from New England Nuclear (Boston, MA), and corticosterone standard from Sigma (St. Louis, MO). This assay and the cortisol assay (Micromedic, Horsham, PA) have been described in detail elsewhere (24). Plasma T_4 and T_3 were assayed using solid-phase RIA kits (Diagnostic Products, Los Angeles, CA) (19, 25). Plasma testosterone was assayed using a solid-phase RIA kit from Immuchem (Carson, CA). All assays (except corticosterone) used human plasma in the standards, and the assays were validated by showing that dilutions of hamster plasma paralleled those of both the standards and the human plasma pools provided with the kits. In addition many different treatments (e.g., short photoperiod in the current experiment) had the expected effects on hormone levels (i.e., very low testosterone levels) (24). The RIA data were analyzed with a weighted least-squares regression program to determine plasma concentrations (27).

All samples from this study were run in the same assay. For corticosterone the minimum detectable dose was 0.34 μg/dl and the intra-assay variability was 10.2% CV at 0.83 μg/dl and 6.0% at 2.48 μg/dl. The cortisol assay had a minimum detectable dose of 0.03 μg/dl, and the intra-assay variability was 6.0% at 0.11 μg/dl and 3.2% at 0.90 μg/dl. The T_4 assay had a minimum detectable dose of 0.03 μg/dl and intra-assay variabilities of 8.9% at 0.67 μg/dl and 4.9% at 2.69 μg/dl. The T_3 assay had a minimum detectable dose of 8.63 ng/dl with intra-assay variabilities of 5.3% at 23.5 ng/dl and 4.1% at 54.3 ng/dl. Finally, the minimum detectable dose for the testosterone assay was 0.12 ng/ml, and the intra-assay variabilities were 10.8% at 0.62 ng/ml and 7.3% at 2.75 ng/ml.

Autopsy results were analyzed using Student's *t* tests. The hormone data were analyzed statistically in two ways. First, the data were analyzed by analysis of variance (ANOVA) to look for overall differences in hormone levels on the two photoperiods, whether the hormone levels varied during the day, and finally whether the hormone patterns were different in the two groups (15). Then the data were analyzed using periodic regression (PR) to look for significant sinusoidal rhythms with periods of 24 or 12 h (6). As explained previously (22), PR is equivalent to ANOVA except that ANOVA uses a linear model to test for differences, whereas PR uses a model composed of a series of sine curves. The PR is a more powerful test for sinusoidal rhythms, but it is sensitive to deviations from a sinusoidal shape. Therefore, ANOVA will be more effective in analyzing nonsinusoidal rhythms. An advantage of PR is that it allows one to locate the peaks of the fitted sine curves, the acrophases, so that the phases of two rhythms can be compared. The acrophase data are presented as means ± SD, and all other results are presented as means ± SE. All probabilities are two-tailed.

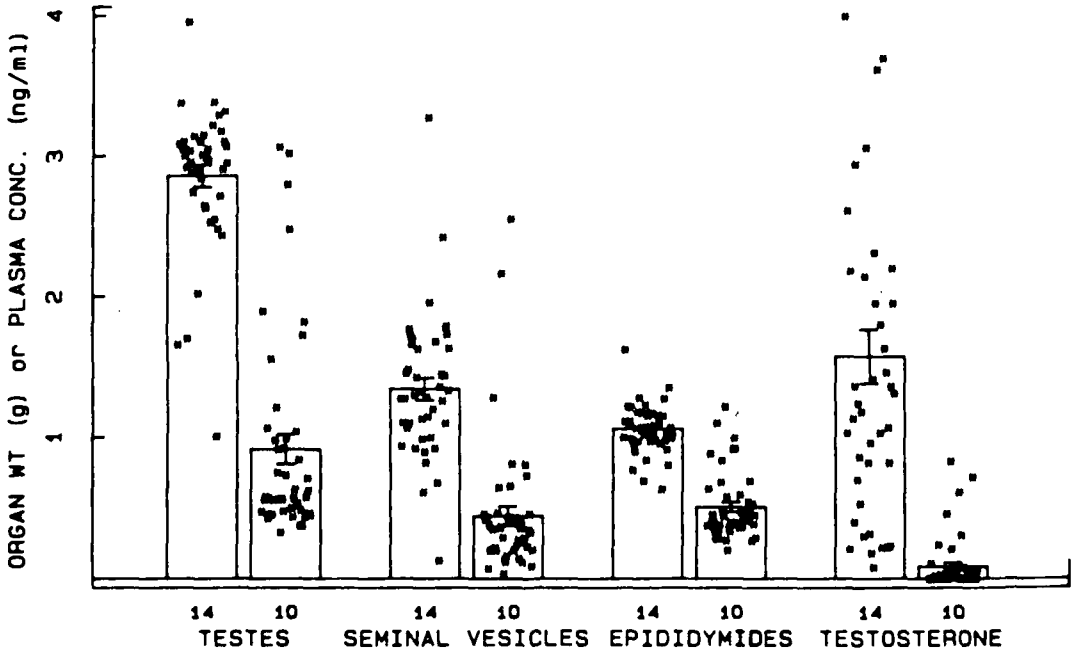

FIG. 1. Testis, seminal vesicle, and epididymis weight and testosterone concentration in hamsters on long (14) and short (10) days. Short days produced lower paired testes, seminal vesicle, and epididymis weights. Testosterone concentrations were also suppressed on short days with low testosterone levels on long days occurring at nadirs of testosterone rhythm. *Bars* represent means ± SE, and *points* represent all individual values.

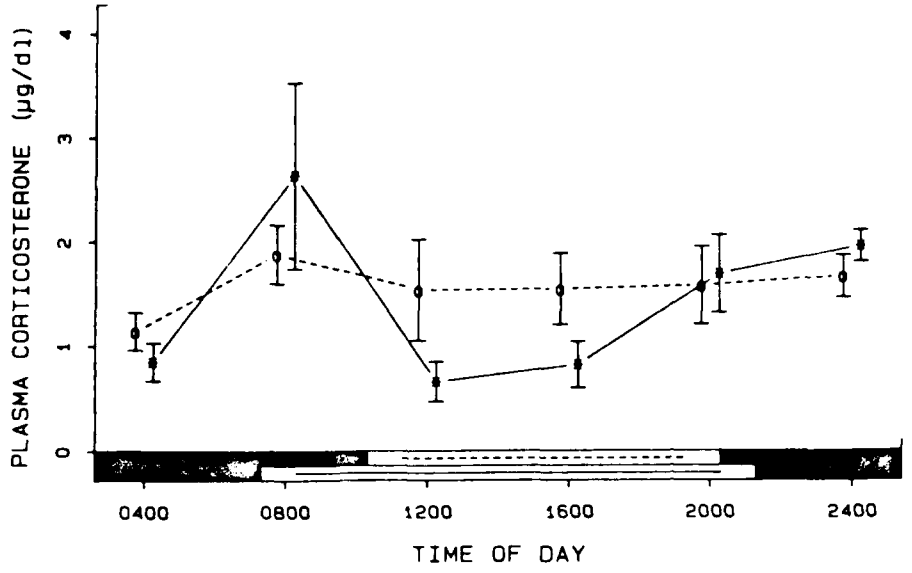

FIG. 2. Daily patterns of plasma corticosterone in hamsters on long and short days. Significant corticosterone rhythm was present on long but not short days. Means ± SE are presented for 6-7 samples per time on long days and 7-8 samples per time on short days. Figures 2-8 present other hormone levels for same samples and have same format. *Solid line*, 14:10-h light-dark cycle; *dashed line*, 10:14-h light-dark cycle.

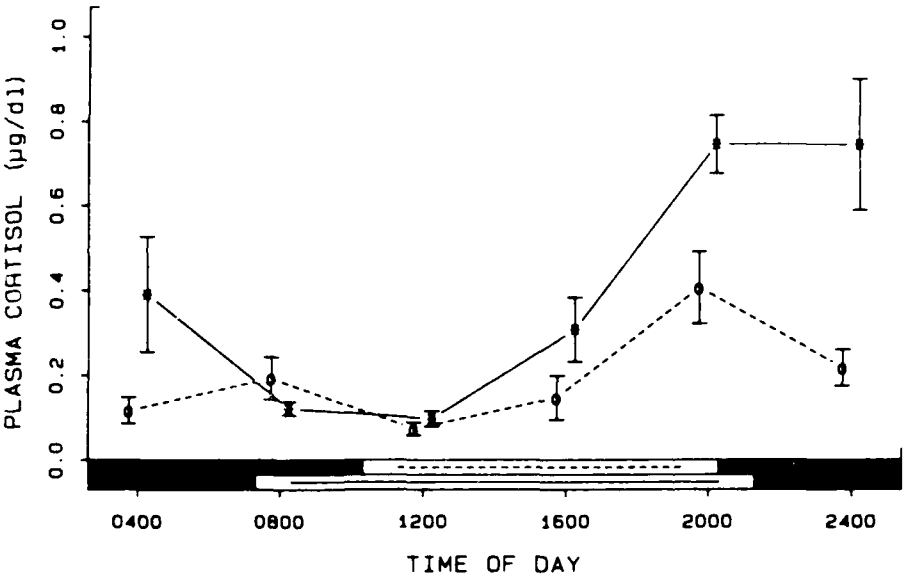

FIG. 3. Daily patterns of plasma cortisol in hamsters on long and short days. Significant cortisol rhythms were present on both day lengths; however, cortisol levels were lower on short days, and amplitude of its rhythm was suppressed. *Solid line*, 14:10-h light-dark cycle; *dashed line*, 10:14-h light-dark cycle.

RESULTS

Autopsy data. As expected, most hamsters on short days (10:14-h LD) had regressed gonads and accessory sex glands (Fig. 1). Short days produced smaller testes (t = 15.75, df = 46, $P < 0.001$), smaller seminal vesicles (t = 8.31, df = 84, $P < 0.001$), smaller epididymides (t = 9.83, df = 86, $P < 0.001$), and lower plasma testosterone levels (F = 117.03, df = 1,73, $P < 0.001$). However, 15% of the short-day hamsters had large testes, seminal vesicles, and epididymides (Fig. 1). Nonetheless, these hamsters had very low levels of testosterone, similar to other hamsters on short days. Thus, although these hamsters appeared to have active reproductive organs, their testosterone levels suggested that they were reproductively inactive, and their data were included in the statistical analyses with the other hamsters on short days. On the other hand, all but one of the hamsters on long days had functionally active testes and enlarged seminal vesicles. This hamster had high levels of testosterone and enlarged epididymis despite having regressed testes and seminal vesicles. Thus the autopsy data demonstrated that the short photoperiod we used produced gonadal regression and that the long photoperiod maintained testicular function.

Glucocorticoid hormones. There was no difference between the two photoperiods in overall corticosterone or total glucocorticoid concentrations, but cortisol levels were higher on long than short days (ANOVA F = 25.21, df = 1,67, $P < 0.001$). There was a corticosterone rhythm on long days (Fig. 2; PR F = 5.34, df = 2,31, $P < 0.02$) with an acrophase just after midnight (0016 ± 0104 h), but there was no corticosterone rhythm on short days. Plasma cortisol was rhythmic on both long (PR F = 24.10, df = 2,33, $P < 0.001$) and short (PR F = 5.10, df = 2,39, $P < 0.025$) days (Fig. 3), but the patterns of the

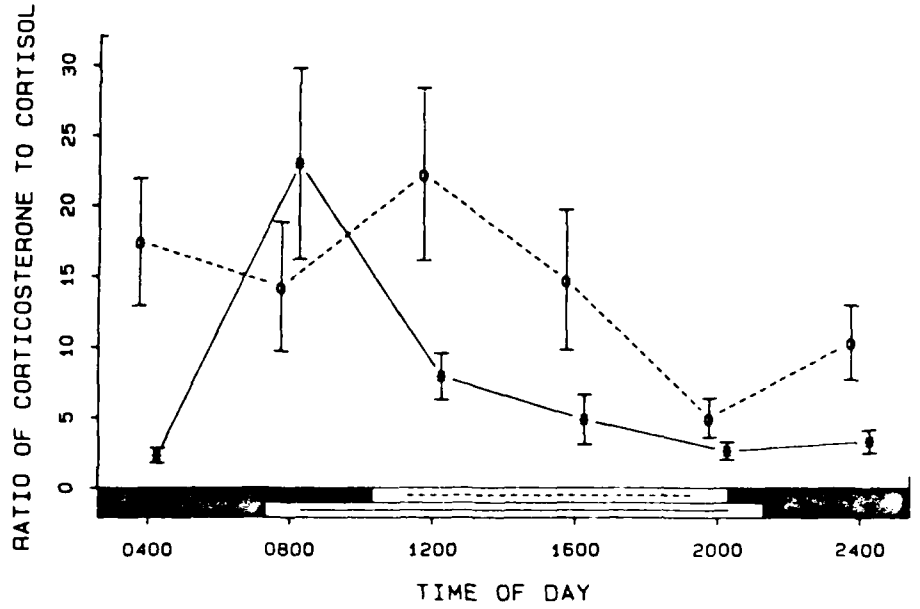

FIG. 4. Daily patterns in the ratio of corticosterone to cortisol in hamsters on long and short days. Significant rhythm in this ratio was present on long but not short days. Ratio of corticosterone to cortisol was lower on long days due to elevated cortisol levels. *Solid line*, 14:10-h light-dark cycle; *dashed line*, 10:14-h light-dark cycle.

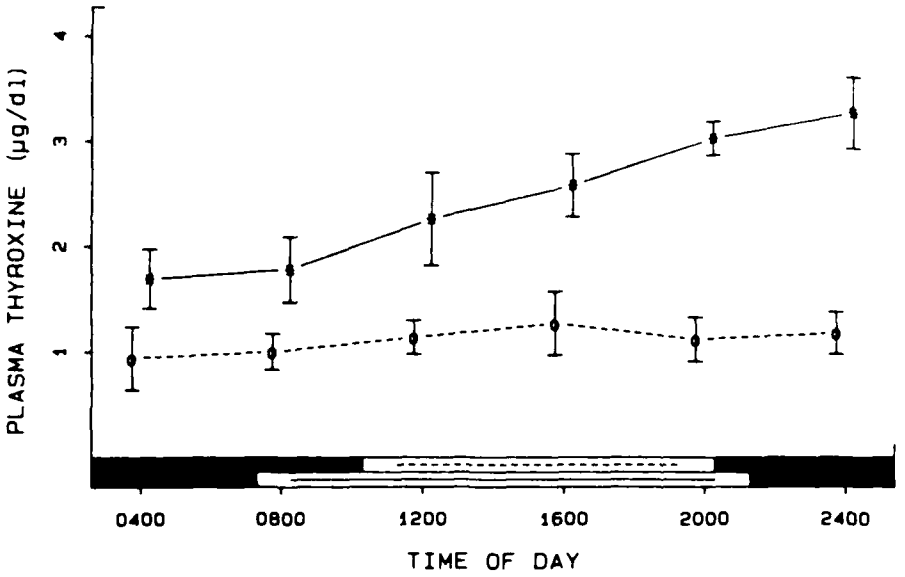

FIG. 5. Daily patterns of plasma thyroxine in hamsters on long and short days. Short days lowered thyroxine levels and eliminated thyroxine rhythm found on long days. *Solid line*, 14:10-h light-dark cycle; *dashed line*, 10:14-h light-dark cycle.

rhythms were different (ANOVA $F = 4.60$, df = 5,67, $P < 0.005$). The acrophases of both rhythms occurred at ~2200 (short days: 2113 ± 0104; long days: 2219 ± 0034), but the amplitude of the cortisol rhythm on short days (0.10 ± 0.03) was significantly less than that on long days (0.37 ± 0.05; $t = 6.51$, df = 74, $P < 0.001$). There was much more corticosterone than cortisol on both photoperiods (short days: ANOVA $F = 89.32$, df = 1,76, $P < 0.001$; long days: ANOVA $F = 73.39$, df = 1,63, $P < 0.001$), and thus the results for total glucocorticoids paralleled those for corticosterone. The finding that plasma corticosterone levels were higher than those of cortisol contrasts with the generally held view that cortisol is the predominant glucocorticoid in hamsters. On long days there was a significant interaction when corticosterone and cortisol were analyzed together (ANOVA $F = 3.40$, df = 5,63, $P < 0.02$), which indicated that the patterns of corticosterone and cortisol were significantly different on this photoperiod. This was supported by the presence of a rhythm in the ratio of corticosterone to cortisol on long days (Fig. 4; PR $F = 7.28$, df = 2,31, $P < 0.01$), which indicated that corticosterone and cortisol could be differentially secreted into and/or cleared from the plasma of hamsters. The ratio of corticosterone to cortisol was lower on long days than short days (ANOVA $F = 7.45$, df = 1,61, $P < 0.02$) because of the elevated cortisol levels on long days.

Thyroid hormones. Both T_4 and T_3 levels were higher on long days than on short days (Figs. 5 and 6; T_4: ANOVA $F = 67.56$, df = 1,70, $P < 0.001$; T_3: ANOVA $F = 25.25$, df = 1,78, $P < 0.001$). As in rats, plasma T_4 levels were 30–70 times higher than T_3 levels (Fig. 7). The T_4 to T_3 ratio was significantly higher on long days (ANOVA $F = 27.41$, df = 1,78, $P < 0.001$), which indi-

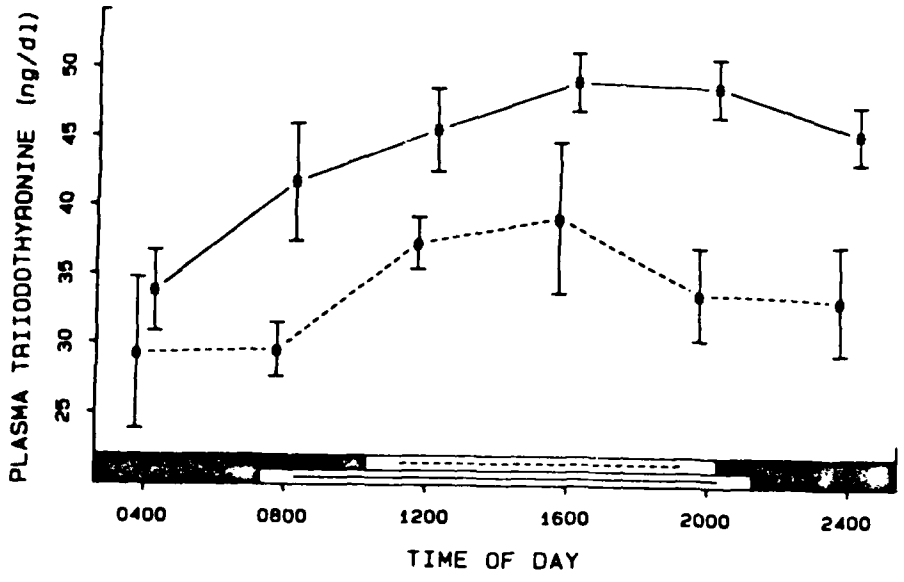

FIG. 6. Daily patterns of plasma triiodothyronine in hamsters on long and short days. Triiodothyronine (T_3) levels were lower on short days. Significant T_3 rhythm was present on long days but neither analysis of variance nor periodic regression detected any significant differences in T_3 levels in hamsters on short days. *Solid line*, 14:10-h light-dark cycle; *dashed line*, 10:14-h light-dark cycle.

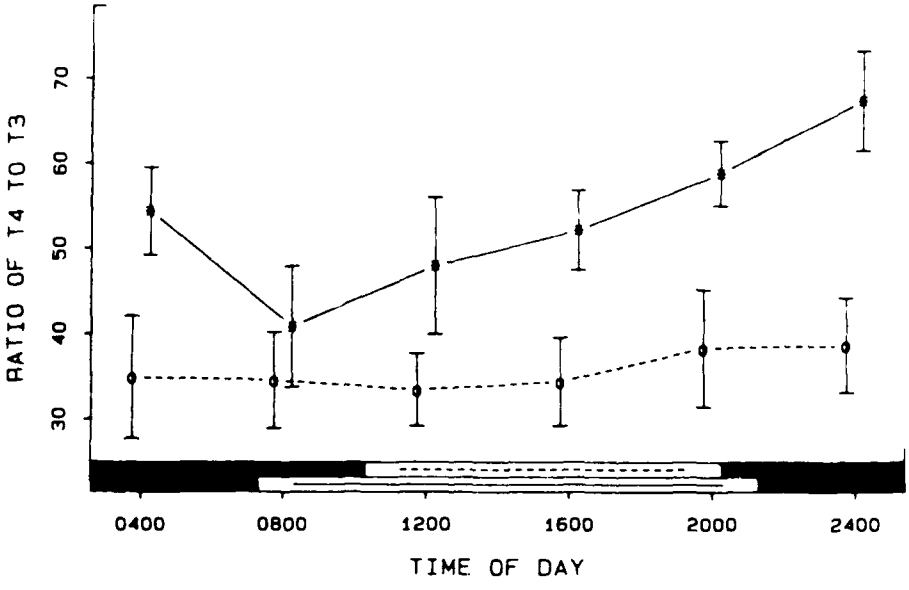

FIG. 7. Daily patterns in ratio of thyroxine (T_4) to triiodothyronine (T_3) in hamsters on long and short days. Ratio was rhythmic on long but not short days. T_4-to-T_3 ratio was lower on short days, which indicated that T_3 was preferentially maintained when both T_4 and T_3 were suppressed by short days. *Solid line*, 14:10-h light-dark cycle; *dashed line*, 10:14-h light-cycle dark.

cated that plasma levels of T_3 were preferentially maintained when overall thyroid hormone levels fell. Significant plasma T_4 (PR $F = 8.64$, df = 2,39, $P < 0.002$) and T_3 (PR $F = 7.61$, df = 2,39, $P < 0.005$) rhythms were present on long days but not on short days. The acrophase of the T_4 rhythm on long days occurred at 2000 (2009 ± 0054) just before the onset of dark, whereas the T_3 rhythm on this photoperiod peaked near 1700 (1711 ± 0058). The T_3 rhythm peaked before the T_4 rhythm on long days ($t = 3.15$, df = 76, $P < 0.01$), and as might be expected the ratio of T_4 and T_3 was rhythmic (PR $F = 5.16$, df = 2,39, $P < 0.025$) and peaked at 2230 (2220 ± 0113). The plasma T_3 levels on short days showed no significant rhythm.

Testosterone. Plasma testosterone levels were much higher on long days than on short days (Fig. 8; ANOVA $F = 117.03$, df = 1,73, $P < 0.001$). More than one-half of the plasma samples on short days had undetectable amounts of testosterone. Thus a floor effect may have biased the analyses that included testosterone levels on short days. A significant bimodal testosterone rhythm was present on long days (PR $F = 22.91$, df = 2,37, $P < 0.001$), which peaked at 0800 and 2000.

DISCUSSION

The data indicated that adrenal, thyroidal, and testicular hormone rhythms were suppressed on the short photoperiod. Plasma cortisol was the only hormone that showed a significant circadian rhythm on the short photoperiod, and even its amplitude was significantly lower than the cortisol rhythm on the long photoperiod. In addition short days also produced lower overall levels of cortisol, thyroid hormones, and testosterone. Although others have not examined thyroid hormone and testosterone rhythms on long and short days, they have pre-

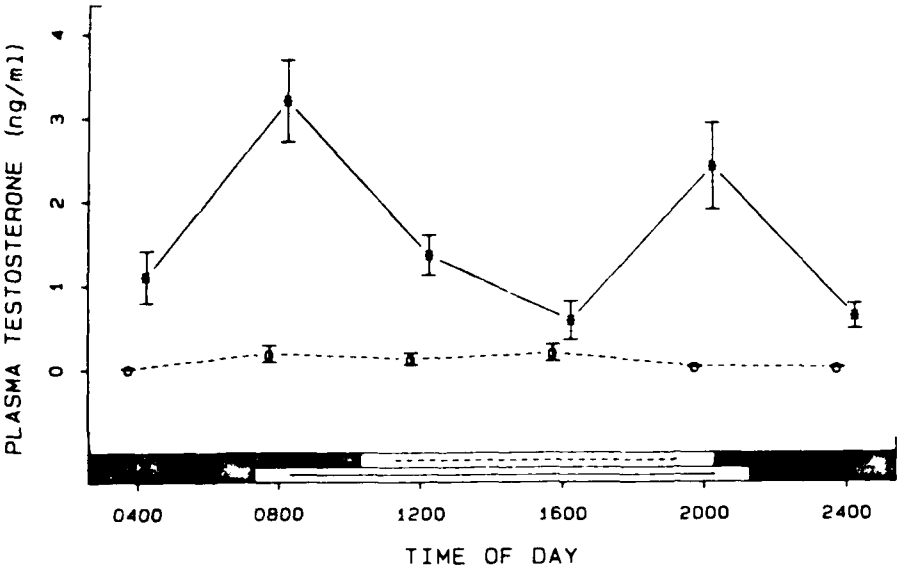

FIG. 8. Daily patterns of plasma testosterone in hamsters on long and short days. Significant bimodal rhythm was present on long days but no rhythm was detectable on short days. Testosterone levels were very low on short days and more than half of samples on short days had testosterone levels that were below minimum detectable dose. *Solid line*, 14:10-h light-dark cycle; *dashed line*, 10:14-h light-dark cycle.

viously reported that levels of these hormones are suppressed on short days (5, 35). The presence of nondetectable hormone levels was only a problem in the analysis of plasma testosterone levels on the short photoperiod. Thus there appeared to be sufficiently high mean levels of the other hormones on the short photoperiod to allow detection of any significant rhythms that might have been present. It is unlikely that the lack of rhythms on the short photoperiod was due to desynchronization in hormone rhythms among individual hamsters, because behavioral rhythms and their controlling oscillators in hamsters are entrained on this photoperiod.

Where comparable data are available, our results are similar to those obtained by others. De Sousa and Meier (9) measured plasma cortisol to assess glucocorticoid rhythms in hamsters on long and short days, and they found a cortisol rhythm in male hamsters on long days but not on short days. Albers et al. (2) found corticosterone and cortisol rhythms in hamsters on long days that were similar to ours, although there was no significant difference between the patterns of the two glucocorticoids in their study. The lack of a significant difference in either mean level or in the patterns of the two glucocorticoids may have been because only four hamsters were sampled at each time point (2). Finally, Vriend reported T_4 and T_3 rhythms in male hamsters on long photoperiods that were very similar to ours (34).

Our data on the relative amounts of corticosterone and cortisol in the plasma of hamsters conflicts with the generally held view that cortisol is the predominant plasma glucocorticoid in hamsters. The first studies of hamster glucocorticoids were reported in 1959 and showed that cortisol was the predominant glucocorticoid secreted by the hamster adrenal cortex (30, 31). However, the blood samples in these studies were collected from venous cannulas under Nembutal anesthesia immediately after cannulation surgery. This was necessary to collect samples with sufficiently high concentrations of steroid to measure by the methods of that time, but the results obtained under such conditions cannot reflect basal steroid secretion patterns as was recognized by the developer of adrenal vein cannulation (7). These results were confirmed in 1965 using very similar techniques, but this study found that cortisol could represent as little as 41–61% of the total glucocorticoid secretion under some conditions (11). In the early 1970s, another group examined plasma glucocorticoid levels in basal samples from decapitated rats (12, 13), but these researchers used a competitive protein binding assay that does not distinguish between cortisol and corticosterone. Nonetheless, they used cortisol standards based on the earlier work and reported their results as plasma cortisol concentrations. These studies and others using competitive protein assays (16) represent accurate assessments of total plasma glucocorticoid activity but clearly not plasma cortisol concentrations. However, plasma cortisol and corticosterone concentrations can now be measured with highly specific RIAs that have very little cross-reactivity. If a cortisol assay is used to assess glucocorticoid activity in the mistaken belief that cortisol is the predominant glucocorticoid in hamsters (9, 37), researchers may be missing important changes in corticosterone levels or total glucocorticoid activity. Finally, we are not aware of any available data on the relative biological potencies of these two glucocorticoids in hamsters. Thus it is currently impossible to assess the relative contributions of the two glucocorticoids to total plasma glucocorticoid activity in the hamster.

Because of the suppression of all hormone rhythms on short days and the decline in mean levels of most hormones, the relationships between these changes is uncertain. One possibility is that the fall in thyroid hormone levels is the primary effect of short days, and the other changes in hormone levels and rhythms are secondary. One of us has shown previously that the amplitude of the plasma corticosterone rhythm in both intact and hypophysectomized rats is dependent on the presence of thyroid hormones (21, 23). This effect of thyroid hormones has been confirmed by others (20). In one of these studies with rats (21), the replacement of T_4, which

reversed the effects of thyroidectomy and restored the corticosterone rhythm, did not produce either T_4 or T_3 rhythms. Thus rhythms of plasma T_4 and T_3 are not required for the expression of the plasma glucocorticoid rhythm. However, it is clear that hypothyroidism does not result in complete aperiodicity because the plasma cortisol rhythm was present on short days in the current experiment and behavioral rhythms can persist in the hypothyroid condition (19, 20). Thyroid hormones have also been implicated in testicular photoperiodic responses (35).

It has been known that there are photoperiodic responses of the thyroid gland to day length in many species. More recently, it has been shown that short days, afternoon melatonin injections, and blinding all cause decreases in plasma thyroxine in hamsters (33, 36). In another study, Petterborg et al. (25) reported thyroid hormone levels very similar to ours in hamsters on similar photoperiods. However, they found a decrease in T_4 and not in T_3 levels on a 10:14-h photoperiod compared with a 14:10-h photoperiod. This points to one problem in using samples at a single time of day to characterize endocrine states under different treatments. At some times of day (i.e., 0400, 1200, and 1600) we did not find significant differences between T_3 levels on long and short days, but at the other times of day (i.e., 0800, 2000, and 2400) T_3 levels were significantly higher on long days (Fig. 6). Thus, unless the changes in overall thyroid status are much larger than the circadian variation in thyroid hormone levels, one must be careful in studying photoperiodic effects by drawing blood samples at only one time of day.

Although much data are available suggesting that thyroid hormones may be important in mediating other photoperiodic effects, it is clear that testosterone might also have such a role. Testosterone clearly responds to photoperiod in a wide variety of vertebrate species. Testosterone can also affect plasma glucocorticoid levels in hamsters (12) and thyroid function in rats (3, 8). Finally, testosterone levels are known to affect behavioral circadian rhythms (28), and orchidectomy may suppress plasma cortisol rhythms in monkeys (C.J. Smith and R.L. Norman, personal communication). Thus a case could also be made that the primary effect of photoperiod on endocrine systems is on the testicular hormone axis, and the effects of photoperiod on adrenal and thyroid hormone levels and rhythms are secondary to low testosterone levels.

Finally, there is less evidence to suggest that the primary endocrine effect of photoperiod is on the adrenal glucocorticoids. The glucocorticoids can affect both thyroid hormone and testosterone levels (17, 38). There are conflicting data as to whether adrenal rhythms are important in regulating behavioral rhythms that presumably reflect the timing of circadian oscillators. One study has shown that glucocorticoid injections can entrain locomotor activity rhythms in rats (14), whereas another study in hamsters showed that rhythmic glucocorticoid infusions do not entrain activity rhythms (2). Thus the relationships between adrenal hormones and other rhythms is not as clear as those for thyroid and testicular hormones.

It is clear that there can be many interactions among the three hormone systems that we studied and that there is insufficient data to determine the mechanisms by which photoperiod might affect them. Yet another possibility is that all three effects are independent of each other and dependent, rather, on some common factor that directly affects all three. Melatonin secretion by the pineal gland is higher in hamsters on short than on long days. It has been reported that melatonin can alter glucocorticoid responses to adrenocorticotropic hormone (29) and suppress plasma levels of thyroid hormones (34) and testosterone (32). Another possible common factor is the pituitary gland that secretes the trophic hormones that maintain the function of all three endocrine systems that we studied. Hypophysectomy is known to alter locomotor activity rhythms in hamsters (40), and short days might have general effects on the pituitary that suppress trophic hormone secretion and the hormonal rhythms we observed. However, it should be kept in mind that none of these studies isolated the effects of melatonin or hypophysectomy to a single hormone system.

In summary the data indicate that significant rhythms in plasma glucocorticoids, thyroid hormones, and testosterone are present in hamsters on long days. However, on short days, the amplitudes of these rhythms are suppressed, and the rhythms disappear except for plasma cortisol. These results in hamsters pose a problem for the application of Meier's internal coincidence model as originally formulated (18), which depended on the phase relationship between two hormone rhythms for the mediation of photoperiodic effects. The absence of hormone rhythms in hamsters on short photoperiods makes it difficult to explain how photoperiodic (i.e., short day) effects might be mediated by such rhythms. However, more recent applications of Meier's model have emphasized that endocrine rhythms may only be peripheral manifestations of neural clocks and that the phase relationship between these clocks is what is crucial in producing photoperiodic effects (9). Meier hypothesizes that such clocks may be affected by hormone rhythms but that their photoperiodic actions may be mediated by mechanisms other than circadian endocrine rhythms.

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Gonadal Function During Prolongation of Life Produced by Constant Light in Hamsters With Heart Failure[1]

W. N. TAPP, B. H. NATELSON, C. KHAZAM AND J. E. OTTENWELLER

Primate Neuro-behavioral Unit, VA Medical Center, East Orange, NJ and Department of Neurosciences, New Jersey Medical School, Newark, NJ

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TAPP, W. N., B. H. NATELSON, C. KHAZAM AND J. E. OTTENWELLER. *Gonadal function during prolongation of life produced by constant light in hamsters with heart failure*. PHYSIOL. BEHAV. 40(2) 243–246, 1987.—In a prior experiment, we showed that living in constant light prolonged the lives of cardiomyopathic (CM) hamsters dying of heart failure. One possible explanation for this therapeutic effect related to the physiological effects of living in short and long days. The control hamsters for our constant light experiment lived in light/dark 12:12, a short day regimen which produces inhibitory effects on the gonadal function of healthy hamsters. To see whether the CM hamster responded to short days like healthy hamsters, we (a) measured gonadal and seminal vesicle mass and plasma testosterone at 1 year of age in CM hamsters raised in the 2 light conditions and (b) assessed testicular size repeatedly over the lives in a second group of hamsters raised in the 2 light conditions. As in healthy hamsters, we found gonadal function in CM hamsters to be greatly inhibited by LD 12:12. Importantly, we replicated our finding that living in constant light prolongs the life of CM hamsters in heart failure. We also found that the stress and trauma inherent in our repeatedly using surgery to assess testicular size acted as an additional risk factor controlling the lifespan of these animals. Our findings suggest that a photoperiodic effect of day length should be considered as one possible explanation of the mechanism of prolongation of life produced in hamsters with heart failure by constant light.

Heart failure Stress Non-pharmacological therapy Gonadal function Photoperiodism

THE diagnosis of congestive heart failure (CHF) carries a dire prognosis. More than 50% of all patients with CHF die within 5 years despite receiving the best possible medical treatment [17]. Because of this outlook, it is critical that new therapeutic avenues for the treatment of CHF be developed. We have recently shown that an environmental prescription, living in constant light, extends life in cardiomyopathic (CM) hamsters that suffer early death from chronic progressive heart failure [19]. Heart failure in CM hamsters [2] resembles heart failure in humans following a diverse number of cardiac insults [4]. CM hamsters in constant light lived up to 25% longer than CM hamsters that lived in cycles of 12 hr of light and 12 hr of dark [19]. However, the mechanism of this striking therapeutic effect is unclear.

One possibility is that the difference in lifespan is related to the physiological effects of day length. Short days, like those that occur naturally in late fall, inhibit gonadal function and prepare hamsters for hibernation. Long days stimulate reproductive function and prepare hamsters for active summer lives. The long day response requires at least 12.5 hr of light per 24 hr. Less than 12.5 hr produces the short day response [5]. We used a 12 hr day in our original study. Therefore, the difference in the long day and short day responses might be important for understanding why CM hamsters in constant light lived longer in our original study. However, we cannot be certain that CM hamsters undergo the same short day response that healthy hamsters undergo. Therefore, the purpose of these experiments was to examine testicular function in CM hamsters living in constant light and light/dark 12:12 (LD). In addition, we took this opportunity to attempt to replicate our original study.

METHOD

Experiment 1

This experiment measured indices of reproductive function in 12 month CM hamsters that had been raised in LD or constant light. Male CM hamsters (BIO 14.6 from Bio-Research, Cambridge, MA), 1–2 months of age, were randomly assigned to live in constant light or LD 12:12 with free access to food and water; average light intensity at the cage floor was 1700 lux during light and less than 0.1 lux during dark. At 12 months of age—one month short of the median survival time of CM hamsters living in LD in our colony

[1]Requests for reprints should be addressed to B. H. Natelson, M.D., Primate Neuro-behavioral Unit (127A), VA Medical Center, East Orange, NJ 07019.

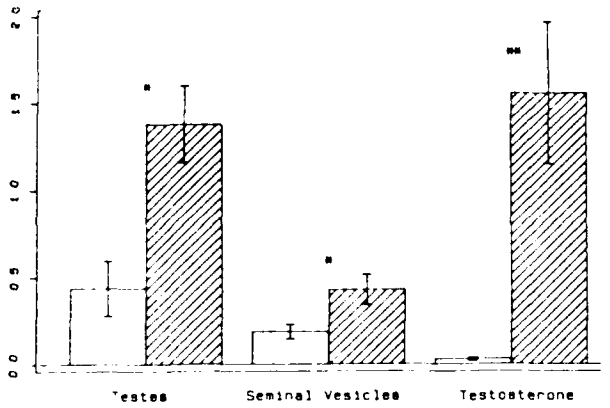

FIG. 1. Mean testes weight per 100 g body weight, mean seminal vesicles weight per 100 g body weight and plasma testosterone (pg/ml) for hamsters in LD 12:12 (open bars; n = 5) and in constant light (hatched bars; n = 6). Vertical bars depict standard error of the mean. The LD housed hamsters have reproductive indices in the non functional range. $^{*}p < 0.05$, $^{**}p < 0.01$

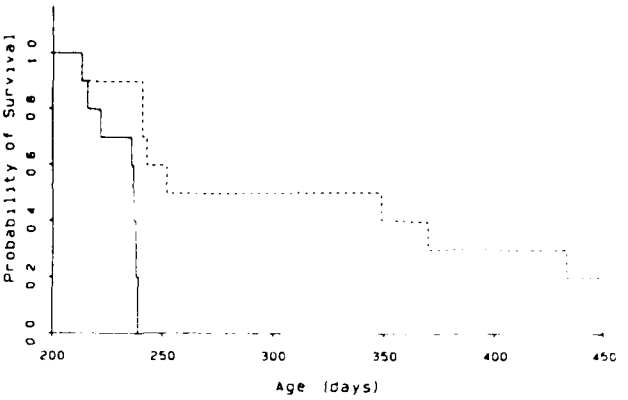

FIG. 2. Kaplan-Meier survival curves [9] for hamsters housed in constant light (dashed line) and in LD (solid line). The experiment began with 10 hamsters in each group. Life in constant light shifted the survival curve of death due to CHF in these CM hamsters significantly.

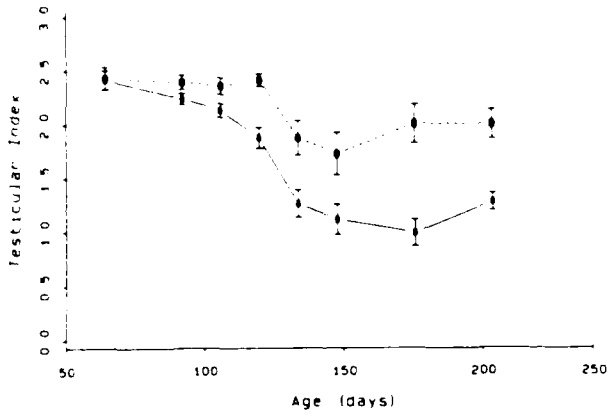

FIG. 3. Testicular index, a derived measure of testicular mass [16], for CM hamsters in constant light (dashed line) and in LD (solid line). vertical bars depict standard error of the mean. Data have been plotted until the first mortality depicted in Fig. 2. LD-housed animals show significantly smaller testes than those maintained in LL, their testes size were in the nonfunctional range since a testicular index of about 1 represents reproductively incompetent gonads [16].

[12]—hamsters were decapitated and trunk blood was collected into heparinized tubes and centrifuged for collection of plasma which was frozen until assay. Testes and seminal vesicles were removed and weighed; seminal vesicles are a target organ of testosterone and therefore a functional index of fertility. Subsequently, plasma testosterone concentrations were measured in 25 μl of unextracted plasma using a solid-phase radioimmunoassay kit (Immuchem; Carson, CA).

Experiment 2

This experiment measured testes size at periodic points throughout the lives of CM hamsters raised in LD (n = 10) or in constant light (n = 10) and maintained as described above; hamsters were placed in their respective lighting conditions at 6 weeks of age. To assess testes size, hamsters were anesthetized with Metafane, and the testes were externalized through incisions in the overlying skin. Length and width of the testes were measured using calipers, and the incisions were sutured closed and cleaned with Prepodyne. Testes measurements were used to derive a standardized testicular index [16] [left(length · width) + right(length · width)] [2 · body weight in g]. Testes were first measured when the hamsters were 8 weeks old, and the measurements were repeated bi-weekly until the hamsters were 5 months old. Thereafter, measurements were made on a monthly basis. Hamsters were weighed periodically throughout their lives. The date the hamster succumbed to its disease was recorded.

RESULTS

Experiment 1

Figure 1 shows that hamsters raised in constant light had significantly higher plasma testosterone concentrations and significantly heavier testes and seminal vesicles than hamsters raised in LD, $t(10) = 3.69$, 2.76, and 2.35 respectively; $p < 0.01$, 0.02 and 0.05 respectively. These results suggested that CM hamsters undergo gonadal regression in short days. However, if this study had been done on healthy hamsters living in short days for as long a time as this, testicular function would have returned toward normal because of spontaneous recrudescence [13,14]. Thus, the results in this experiment may have been confounded by the co-existence of severe disease in these 12 month old animals. Because of this, we performed the second experiment which allowed us to follow testicular size over an individual hamster's lifespan.

Experiment 2

Survival curves of hamsters in the two lighting conditions revealed that hamsters in constant light lived significantly longer than those in LD ($p = 0.00014$; log-rank test [9]; see Fig. 2). Median lifespan was 7% longer for hamsters in con-

stant light (254 days) than in LD (237 days). Remarkably, when 100% of the LD hamsters had died, 90% of the hamsters in constant light were still living. Deaths showed a distinct tendency to cluster in the period of time following testes measurements: 15 of the 18 animals that died during the study did so in the first 2 weeks after measurement (binomial $p = 0.006$).

Figure 3 reveals that hamsters in constant light had significantly larger testes indices than those in LD (analysis of variance for repeated measures, $F(1,18) = 17.5$, $p < 0.01$); the difference between the groups became significant at day 92 (Dunn's post-hoc test, $p < 0.01$). Testes index declined over time in both groups, $F(7,126) = 40.8$, $p < 0.0001$, but the decline was greater for LD hamsters than for those raised in constant light, $F(7,126) = 6.6$, $p < 0.001$. Concurrent with these changes in testes index was a significant difference in body weight (average weight just before the first death was 141 ± 3 (SEM) g in LD and 131 ± 3 g in constant light, $t(18) = 2.44$, $p < 0.025$. The changes in testes index were not due to differences in body weight because a reanalysis of testes size without the correction for body weight produced the same results. Testes size at the last measurement prior to death did not predict anything about lifespan within a light condition. CM hamsters dying early in constant light did not have smaller testes than those that survived several months longer.

DISCUSSION

This study represents an important replication of our previous work: hamsters succumbing to early death from chronic, progressive heart failure live longer when raised in constant light than they do when raised in LD. However, the stress and trauma associated with the surgery used to measure testes size appear to have acted as an additional risk factor in this study. Median lifespan for both groups was reduced by more than 100 days from the median lifespan of unoperated CM hamsters in our earlier work [19], and death clustered in the period of time following the surgery required to measure testes size. We have shown that saline supplements in this model also increase risk [19] and that stress itself can precipitate overt signs of CHF in CM hamsters housed in LD [18].

The other purpose of this study was to begin an analysis of possible mechanisms of constant light's effect on longevity by determining whether CM hamsters undergo gonadal regression in the constant light-LD experiment. Both experiments showed that CM hamsters undergo testicular regression in 12 hr of light/24 hr. The first experiment showed that CM hamsters in 12 hr of light had testes sized in the functionally incompetent range while CM hamsters in constant light had large, apparently functional testes. Reinforcing this conclusion, atrophied seminal vesicles and extremely low testosterone levels were found only in LD hamsters. This difference was not due to the existence of overt CHF in LD hamsters. The second experiment showed that LD hamsters had testes indices in the functionally incompetent range by 140 days of age when there were no signs of CHF. In contrast, the indices reflected fully competent testes throughout the lives of hamsters raised in constant light.

The critical question is whether these differences in gonadal function influence survival during heart failure. An examination of the literature found no data to indicate that testicular function would be protective. On the contrary, a number of studies suggest that hypotesticular states are beneficial in certain situations. Loss of testicular function has been associated with increased lifespan in healthy people [7] and animals [1,6], and a number of pathophysiological processes related to the heart (e.g., coronary artery atherosclerosis [10]) occur less often in females than males. These data on normal aging and on disease initiation do not exclude the possibility that improved gonadal function and/or increased testosterone could improve the course of an already established disease process such as CHF. Hypogonadism accompanies the end stages of CHF [20]. Whether treating patients with these problems with testosterone would alter the outcome of their disease is not known, but our data certainly make the question worthy of consideration.

However, alternative possibilities exist to explain life extension in constant light. Testicular function may not affect lifespan at all, or testicular function might actually lessen the protective effect of life in constant light like stress or saline do. Some other effect of long and short day lengths may alter lifespan in CM hamsters. Changes in day length influence other physiological systems besides the reproductive system [3,11]. One such possibility is that light might be acting through the pineal gland. Pineal secretions are sensitive to day length and affect gonadal function [15]. Pinealectomy has been reported to prolong the life of hamsters [8]. Such an explanation obviously warrants further experimental consideration.

As one possible mechanism for life extension in constant light, we originally suggested [19] a photoperiodic effect due to differences in day length. However all the work on the photoperiodic effects has been done in healthy animals, and so we did not know whether the CM hamster would show the same responses to long and short days as the normal hamster. This experiment makes it clear that the CM hamster in LD 12:12 has a gonadal deficit compared to hamsters in constant light. Since gonadal deficits occur in hamsters living in short photoperiods compared to others living in long photoperiods, our findings suggest that a photoperiodic effect of day length is one possible explanation for the results obtained. This information allows us to progress in our analysis of mechanism to determine whether life extension in constant light relates to day length per se or instead to some other property of life in constant light.

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ANOMALOUS RELATIONSHIPS BETWEEN CIRCADIAN RHYTHMS: QUESTIONS ABOUT OSCILLATORS, COUPLING AND ENTRAINMENT

W.N. Tapp*, S.S. Reisman**, R.H. Natelson*, T.A. Pritzel*

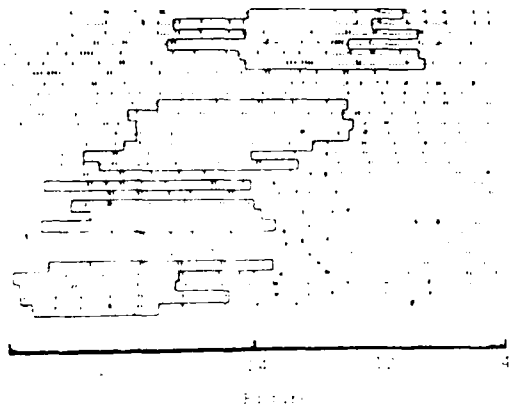
*VA Medical Center and Dept. of Neuroscience, New Jersey Medical School, East Orange, N.J.

**Dept. of Electrical Engineering, New Jersey Institute of Technology, Newark, N.J.

We have studied circadian rhythms of activity, temperature, feeding and performance in rhesus monkeys. During the course of these studies we have observed three types of anomalous relationships between different circadian rhythms within the same subject. These anomalies raise questions about the pacemaker(s) underlying circadian rhythms and the importance of environmental variables other than the light cycle, especially cognitive influences or work demands.

The monkeys in these studies were instrumented for temperature collection through a thermistor implanted in the retroperitoneal cavity. Activity was recorded via a motion detector attached to the sleeve of the monkey's instrumentation vest. Some of the monkeys earned all of their daily food by working on a chained vigilance-discrimination task. In the vigilance task, the monkey had to detect a white cue light that was illuminated at random intervals averaging 2.4 min and press a lever within 10 sec. Immediately after each successful vigilance trial, the monkey received a discrimination trial where the cue light changed to either red or green. The monkey had to press his left lever for a green light and his right lever for a red light. If he successfully completed this choice within 10 sec, he was rewarded with a 750 gm Noyes pellet. Other monkeys only had to press a nosekey to obtain pellets.

Figure 1 shows the first anomaly that we will discuss. The figure displays the feeding record of a monkey that no longer shows a discernible circadian feeding rhythm. The boxes



Figure

that overplot the feeding record show the times when the monkey's temperature exceeded the median daily temperature. (Breaks in the record are due to equipment failure.) The plot shows that the monkey still has a clear freerunning temperature rhythm despite the fact that his feeding rhythm is gone. We have seen seen this phenomenon -- where a monkey loses a detectable circadian rhythm of activity or feeding but still has a circadian temperature rhythm -- in 6 monkeys living in constant light. The duration of these episodes is variable, with the longest lasting 97 days. The onset and end of these episodes appears to be spontaneous in that we cannot discern any external cause for their beginnings and ends.

This pattern could be explained by at least two mechanisms. There could be separate circadian oscillators driving feeding and activity and temperature, and these could be differentially susceptible to disruption by constant light. Alternatively, the same pattern could be produced if constant light had differential effects on the coupling between a single circadian oscillator and feeding, activity and temperature.

In either event it is suggestive that we have never seen the phenomenon in monkeys performing a complex task to earn their food. We have only seen it in monkeys living in constant light and only in monkeys that have ad lib food available via a nosekey. This suggests that cognitive demands and workloads may influence some aspect of the circadian system that prevents this phenomenon.

These findings may be related to reports that the human sleep-wake cycle becomes fragmented when cognitive and social demands are removed. Campbell (1984) reported that when humans were prohibited from most stimulating activities such as watching television, listening to music, reading, etc their sleep shifted from the usual monophasic pattern to a polyphasic pattern with a mean sleep-wake cycle length of 6 hrs. Similar results have been reported in bedrest studies where subjects are not allowed to be isolated from work and other cognitive demands (Nakagawa, 1980). We must consider the possibility therefore, that the monophasic shape of many behavioral rhythms depends upon the influence of cognitive demands and workloads.

The second finding touches on an old debate. Figure 2 shows an example of apparent spontaneous internal desynchronization in a monkey living in constant light and working on the vigilance-discrimination task. The plot labeled

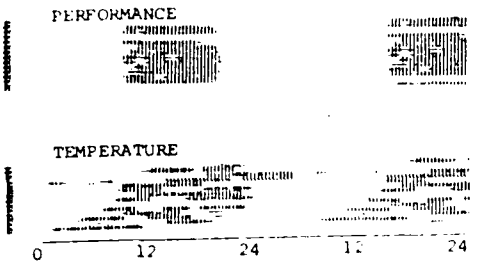


Figure 2

"Performance" shows the pattern of successful trials. Since task trials were presented around-the-clock, the performance plot shows when the monkey chose to work. In this case the monkey exhibits a freerunning performance period of 24 hrs, and a freerunning temperature period of 23.7 hrs. For the sake of space, only a segment of the record is shown here. The total length of internal desynchronization in this case is 27 days. A second similar case has been observed in a monkey with a performance rhythm of 23.7 hrs and a temperature rhythm of 23.1 hrs. This second case lasted for only 16 days.

Spontaneous internal desynchronization has been reported in humans living in constant environmental conditions (Aschoff & Wever, 1976), but a similar phenomenon in animals has been elusive. However, recent authors have challenged the description of internal desynchronization in humans (Zulley and Campbell, 1985). They have suggested that internal desynchronization is an artifact of short sleeps that were discounted in previous analyses of the human data. But this explanation does not account for the desynchronization seen in the monkeys. Successful performance on the vigilance-discrimination task requires that the monkey be awake, and even short sleeps of a few minutes duration would be detected as missed trials. Instead, the monkeys showed steady, successful performance throughout the "active" part of their cycle.

The two cases of monkey desynchronization that we have seen do exhibit several differences from the phenomenon that has usually been reported in humans. First, we have not seen the extreme periods reported in some cases of human desynchronization. Second, it is clear that neither of our monkeys were desynchronized long enough for one rhythm to scan an entire cycle of phases of the other rhythm. Thus, desynchronization in monkeys suggests that the phenomenon is not artifactual, but it does not provide support for multiple oscillators.

Again, it is intriguing to speculate that an anomalous circadian condition -- the elusive spontaneous internal desynchronization -- might be linked in some way to nonlight variables such as cognitive demands or workload. We have only seen this phenomenon in 2 of the 4 monkeys that we have studied with freerunning task.

Other observations point to an important role of cognitive demands and work load on the circadian system. Wirz-Justice and Pringle (1987) recently reported a case of delayed sleep phase and very long days similar to those seen in temporal isolation in a student with no imperative obligations. The notable exception was a period of stable sleep-wake cycles that coincided with his preparations for final exams. As soon as he finished exams, he began to free-run again in a manner reminiscent of people in temporal isolation. This interesting case hints that, in the absence of cognitive demands, the day-night cycle alone may not be sufficient for entrainment.

These observations suggest that workloads and cognitive demands may play a more important role in maintaining the integrity of the circadian system than was previously recognized.

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CIRCADIAN RHYTHMS AND PATTERNS OF PERFORMANCE BEFORE AND AFTER SIMULATED JET-LAG[1]

Walter N. Tapp and Benjamin H. Natelson

Primate Neuro-behavioral Unit, VA Medical Center, East Orange NJ 07019 and Department of Neurosciences, New Jersey Medical School, Newark NJ 07103 USA

Running Title: Circadian rhythms, performance and jet lag in monkeys

Address for correspondence: W.N. Tapp, Primate Neuro-behavioral Unit, New Jersey Medical School, 88 Ross Street, East Orange NJ 07018.

ABSTRACT

We have developed a rhesus monkey model that enables us to investigate physiological rhythms and circadian effects on performance in an integrated framework. Monkeys worked for 8 hrs a day on a chained vigilance-discrimination task, while we recorded activity and temperature rhythms around-the-clock. To test the model, we studied rhythms and performance during entrainment to a 24 hour light cycle and following a 6 hr phase advance. Results from this animal model displayed many of the essential characteristics seen in similar human experiments. During stable entrainment, temperature rhythms peaked in late afternoon, with activity rhythms peaking several hours earlier. Performance exhibited consistent, task-dependent variations over the course of daily sessions. Discrimination peaked at the beginning of the session and vigilance peaked several hours later. Following a 6 hr phase advance, monkeys exhibited transient internal desynchrony with activity resynchronizing faster than temperature. Both vigilance and discrimination were impaired following the phase-shift, with vigilance exhibiting larger magnitude and longer-lasting impairments than discrimination. A second drop in performance was seen 10-14 days after the phase-shift. These data replicate and extend earlier work in humans and show that this model can be used in the study of chronobiological questions that would be too expensive or too impractical to do with humans.

activity circadian rhythms non-human primates phase-shift temperature

Circadian influences on performance are well established. Circadian performance rhythms have been described for a variety of tasks in humans (e.g., 2) and animals (e.g., 5). In addition to producing rhythmic changes in performance, the circadian system can influence the overall level of an individual's performance. The most familiar example of this is the phenomenon of jet-lag that is experienced by people following rapid travel across several time zones. Upon arrival in the new time zone, the transmeridian traveller must phase-shift his circadian rhythms to the new environmental time. Impaired performance on psychomotor and cognitive tasks is characteristic during this period of circadian rephasal, as are fatigue, mood changes and a panoply of physical complaints (6,9,10,18).

Study of circadian influences on performance in humans is complicated by several problems. First, it is difficult and expensive to obtain samples of human performance over many consecutive days. Furthermore, it is difficult to control or account for differences in the sleep-wake and work-rest schedules of subjects prior to entering the study. This limitation is a problem because there are long-term effects of entrainment schedules on the circadian system so that the effects of a particular circadian manipulation may differ depending upon the subject's schedule before entering the study.

In addition, interpretation of human results may be complicated by the presence of long-term trends in performance data due to continuing effects of learning and/or practice on the tasks used in the experiment. Long-term trends due to learning and practice pose special problems for interpreting jet-lag or shift-work data, because circadian phase-shifts have been shown to produce specific deficits in learning (3) and memory (17). It is difficult to be certain, then, that experimental results from tasks that might have a

significant acquisition component would be the same as results on a well-learned, highly-practiced task. While it is possible to minimize these effects with extensive training and practice prior to the start of an experiment, investigators in the field still point to them as serious problems (2). These practice and training effects may be especially important because many of the applied questions concerning jet-lag have to do with deficits in the performance of well-learned, highly practiced tasks.

One solution to some of these problems would be to investigate these questions in an animal model. With animals, it is possible to standardize long-term environmental histories and to provide extensive training and practice on the experimental tasks. However, a critical question is whether the human situation and the animal model are enough alike to expect that the same parameters will be important in circadian influences on human preformance and on animal performance. While there have been reports of circadian performance rhythms in animals (e.g., 5), the human data exhibit important task-dependent differences (7) that have not been explored in animals. Moreover, previous investigations in animals have not examined the relationship between physiological rhythms and performance.

This report describes a monkey model that enables us to examine circadian influences on performance and on other behavioral and physiological processes. It will examine the model during stable entrainment and during experimental jet-lag due to a phase-shift of the light-dark cycle.

METHODS.

Subjects were 6 male rhesus monkeys adapted to living in primate chairs. Each monkey was individually housed in a sound-attenuated, light-tight chamber

that enabled us to maintain strict environmental control. Half of the monkeys (task monkeys) earned all of their food by working 8 hrs/day on a chained vigilance-discrimination task, while the other half (free-feeding monkeys) only had to press a nose key to obtain food at any time during the day or night. All monkeys had free access to water.

Each monkey was instrumented with a retroperitoneal temperature probe interfaced to computerized data collection through a BAT-8 A/D unit (Bailey Instruments; Fairfield, NJ). Access to the probe was prevented by having the monkey wear a vest which attached to the chair's abdominal plate. Activity was monitored from a microswitch sewn into the sleeve of the monkey's instrumentation vest. Activity, temperature and feeding were recorded every 10 min. Additional data on performance were collected from the task monkeys.

In the initial phase of the experiment, monkeys were on a cycle of 12 hr light-12 hour dark (LD 12:12) with lights on from 08:00 to 20:00 h. Task monkeys worked on their task from L+1 to L+8 hrs (09:00 to 17:00 initially). After establishing stable entrainment and performance, monkeys underwent a phase-shift of +6 hours. The phase-shift was accomplished by shortening the monkey's night so that lights-on changed from 08:00 to 02:00. Task availability also shifted by 6 hours to 03:00 - 09:00.

Vigilance-Discrimination Task. Task monkeys had a work panel consisting of a cue light mounted at the monkey's eye level and three levers mounted in front of the monkey. The cue light was covered with a translucent diffuser panel so that each of 3 colored lights could be presented to the monkey without providing any spatial cues.

Figure 1 shows the sequence of a chained vigilance discrimination trial. A trial began with the vigilance component when the cue light was illuminated white. The monkey had 10 sec to detect the light and to respond by pressing

the center lever. The discrimination component began immediately after the monkey successfully completed the vigilance component of the task. The cue light changed from white to red or green, with the sequence of red and green trials being determined according to a random schedule with p = 0.5 for each color. Upon presentation of the red or green cue light, the monkey had 10 sec to press the right or the left lever respectively. If the monkey successfully completed both the vigilance and discrimination parts of the trial, he received one 750 mg bananna-flavored Noyes pellet as a reinforcement. If the monkey made an error at any point in the sequence, either by exceeding the 10 sec time limit or by pressing an inappropriate lever, the trial ended and no pellet was delivered. Trials were presented on a variable-interval schedule with an average inter-trial interval of 2.4 min ($\pm$ 1.38 min SD).

RESULTS

Data from the 20 consecutive days immediately prior to the phase-shift were used as a baseline period in the analysis of rhythms and performance during stable conditions before the phase-shift. During this baseline period, activity and temperature rhythms of all animals showed stable entrainment to the 24 hr light cycle (see Figure 2 for representative example), with temperature peaking in the late afternoon (average time of peak temperature = 17:42 $\pm$ 0.6 hrs SEM) and activity peaking several hours earlier (average time of activity peak = 10:35 $\pm$ 0.7 hrs SEM). The difference in the peak times for the two rhythms was significant ($p<0.01$; Watson-Williams test [11]). Similarly, performance also showed stable daily patterns prior to the phase-shift (see Figure 3 for representative example). Discrimination performance was best at the beginning of the session (average time of peak discrimination

performance = 09:20 ± 0.6 hr SEM). In contrast, vigilance performance improved over the early part of the session, peaking just after mid-session (average time of peak vigilance performance = 14:42 ± 0.6 hr SEM). The difference between the time of peak vigilance and peak discrimination was significant ($p < 0.01$; Watson-Williams test).

Regression analysis revealed modest, but significant, relationships between body temperature and performance. There was a significant inverse relationship between temperature and discrimination performance ($r=-0.215$, $df=2878$, $p << 0.001$), and a significant quadratic relationship between body temperature and vigilance ($r=.172$, $df=2878$, $p << .001$). There was no significant relationship between activity and performance on either task and, interestingly, there was no significant relationship between vigilance performance and discrimination performance.

These stable patterns of entrainment and performance were disrupted by the 6 hour phase advance. Following the 6 hour phase-shift, activity and temperature rhythms required several days to resynchronize to the new light schedule. Complex demodulation (1) at a nominal frequency of 1 cycle/24 hrs with Butterworth low-pass filtering (15) provided estimates of the local phase of the 24 hr rhythms at 10 min intervals. These phase estimates enabled us to track the rephasal of the 24 hr rhythm (see Figure 4 for representative example). Activity reached its new stable phase within an average of 4.1 days ± 0.3 days SEM, while temperature required significantly longer to reach its new stable phase (6.8 ± 0.5 days SEM, $p < 0.025$).

Performance on both tasks was significantly impaired following the phase-shift. Maximal impairment of vigilance averaged 49.1% ± 0.8 SEM ($p < 0.001$, Tukey's test; see top panel of Figure 5 for representative example), and vigilance was significantly impaired (at least $p < 0.05$, Tukey's test) for an

average of 5 days $\pm$ 0.57 SEM. Maximal impairment of discrimination averaged 11.3% $\pm$ 0.1 SEM ($p < 0.001$, Tukey's test; see bottom panel of Figure 5 for representative example), and discrimination was significantly impaired (at least $p < 0.05$) for an average of 2.33 days $\pm$ 0.57 SEM. The magnitude of difference in decrement between the 2 modes of performance was statistically significant ($p < 0.01$). A second, significant decline in performance was found between 10 and 14 days post-phase-shift. This second dip in performance lasting 2 days was seen in all three monkeys for both vigilance (average decrement = 27.2% $\pm$ 0.3 SEM, $p < 0.01$) and discrimination (average decrement = 6.6% $\pm$ 0.9 SEM, $p < 0.01$). As had been the case also in the first period of performance impairment, the magnitude of the decrement in vigilance performance was significantly greater than that of discrimination performance ($p < 0.015$).

In addition to the performance impairments seen in speed of response, the monkeys showed significant increases in the number of discrimination errors (choosing the wrong lever) in the first 2 days following the phase-shift. During the 20 days prior to the phase-shift, the task monkeys averaged only 0.239 $\pm$ 0.086 SEM errors/day. But during the two days immediately following the phase-shift, they averaged 7.16 $\pm$ 1.22 SEM errors/day ($p < 0.01$). Similarly, during the second decline in performance, the rate of discrimination errors again increased significantly above the baseline rate (4.83 $\pm$ 1.19 SEM errors/day, $p < 0.01$).

After the phase-shift, the daily patterns of performance were disturbed for several days, only gradually returning to the pre-shift pattern. To assess the return to the pre-shift pattern, we used each monkey's average pre-shift pattern as a template and computed the correlation between the template and performance on each post-shift day (see Figure 6 for representative

example). As it did for mean daily performance, the pattern of discrimination performance returned to normal significantly earlier (4.0 $\pm$ 0.6 days) than the pattern of vigilance performance (6.3 $\pm$ 0.3 days; $p < 0.01$, t-test on days to reach a significant correlation with the template).

The performance deficit was not due to changes in food-motivated behavior following the phase-shift. Free-feeding monkeys that did not have to perform the task to obtain food showed no significant difference between their daily food consumption prior to the shift (200.5 pellets $\pm$ 5.4 SEM) and during the first 5 days after the shift (202.4 pellets $\pm$ 7.3 SEM) or during the first 20 days after the shift (207.1 pellets $\pm$ 4.3 SEM).

DISCUSSION.

Prior to the phase-shift, monkeys showed stable activity and temperature rhythms that were entrained to the 24 hr light cycle and consistent, asymptotic levels of task performance. Temperature rhythms peaked in the late afternoon, while activity peaked somewhat earlier in the day. This pattern is typical of entrained activity and temperature rhythms in humans (8,19) as well as in squirrel monkeys (16). Overall performance on the chained vigilance-discrimination task was clearly asymptotic and virtually error-free, but there were obvious variations in performance over the course of a day's session.

These performance variations followed consistent patterns that were different for the two tasks. Discrimination performance was best early in the morning at the beginning of the session and declined across the course of the day. But vigilance performance began at intermediate levels, rose to peak just after the middle of the day, and then declined in the afternoon. The pattern of discrimination performance was consistent with our initial

hypothesis that discrimination would behave like the more complex, high memory load tasks do in humans. Like the high memory load tasks in humans (6), monkey discrimination performance was inversely related to body temperature.

However, the vigilance task did not fit the prediction for a low memory task in humans. It peaked earlier than would be posited on the basis of the human data (6), and it exhibited a significant quadratic relationship with body temperature rather than the positive linear relationship that was expected. It appeared to be intermediate between the expected patterns for high and low memory loads. Folkard and his colleagues (6) have reported on a task with an intermediate memory load. Peak performance on this task occurred between the times of peak performance for their high and low memory load tasks, and there was no linear correlation between temperature and intermediate memory load performance. Furthermore, we reanalyzed data in a figure presented by Folkard et al. (6) and found a significant quadratic relationship between temperature and performance on the intermediate memory task ($p < 0.05$) over the hours corresponding to the times when the monkeys worked. The fact that the monkey vigilance task appears to behave more like an intermediate memory task than a low memory task may be due to the fact that the current vigilance task does have a discrimination component. Although the monkey's main job in the vigilance task is to detect the trial, he must discriminate and press the middle lever. It may be that simplifying the task by allowing the monkey to press any lever during the vigilance task would yield performance patterns more like those seen in humans on the low memory tasks. In any event, monkeys showed clear, task-dependent differences in the pattern of performance over time and in the relationship of performance to temperature.

The presence of task-dependent differences in monkey performance patterns

is important, because it is the first time such differences have been reported outside of humans. The most frequent explanations of the human time of day data are based on an arousal hypothesis (12) that has been difficult to explore in animals, because the arousal involved is not correlated with typical measures of arousal in animals, such as activity. According to the arousal hypothesis, there is a circadian rhythm of arousal that roughly parallels the temperature rhythm. Performance is related to arousal by an "inverted-U shaped function", and the optimal level of arousal is determined by task complexity, with low arousal being best for high working memory loads and high arousal being best for high memory loads. While the monkey vigilance-discrimination data are consistent with this explanation, further work is necessary to elucidate the contributions of working memory load, arousal, circadian rhythms and other variables to the time- and task-dependent variations in monkey performance.

Phase-shifting the light cycle by 6 hours produced marked changes in circadian rhythms and performance. Both temperature and activity required several days to resynchronize to the new environmental time. Temperature required an average of just under 7 days to resynchronize. This time is comparable to resynchronization times reported for human temperature following a 6 hr phase advance. For example, Wegmann et al. (18) reported that temperature took 7 days to resynchronize in humans following a 6 hr phase advance. But it seems to be somewhat slower than resynchronization of temperature in squirrel monkeys. Wexler and Moore-Ede (21) reported that resynchronization of temperature in squirrel monkeys required about 5 days after an 8 hr phase advance. Importantly, activity resynchronized about 2 days faster than temperature. Thus, the monkeys exhibited transient internal desynchronization similar to that reported by others for humans (4) and

monkeys (14).

The phase-shift produced substantial decrements in performance on both the vigilance and discrimination tasks. Vigilance was affected worse than discrimination. The magnitude of the performance deficit was larger for vigilance than for discrimination, and vigilance was impaired for a longer time, more than 2 days longer than discrimination. Two types of performance deficits were seen. The speed of response decreased in both vigilance and discrimination. Furthermore, the rate of choice errors on the discrimination task (choosing the wrong lever) increased dramatically above their virtually error-free performance rates in the baseline condition. While these tasks cannot be directly compared to the tasks used with humans, the duration of impairment is comparable to that reported in human psychomotor tasks. For example, symbol cancellation (a low memory task) returned to normal within 5 days after a 6 hour phase advance, and digit summation (a high memory task) returned to normal within 3 days following a phase advance (10).

A surprising finding was the occurrence of a second drop in vigilance and discrimination occurring 10-14 days after the phase-shift and lasting 1-2 days depending upon the monkey. This decline was marked by both decreased response speed and increased choice errors. Most human studies have examined daily performance patterns for 10 days or less following phase-shifts. Therefore, if there is an analogous second decline in performance in humans, it is not surprising that the studies that are currently available have not detected it. If a similar secondary drop occurred in human performance following jet lag, it would raise new problems for operational planners who have to contend with the performance effects of phase-shifts in people.

The deficits following the phase-shift were not due to decreased food motivation during resynchronization. Monkeys with free access to food showed

no significant change in food consumption during the first 5 days or the first 20 days following the phase-shift.

It is also difficult to ascribe the performance deficits to the transient internal desynchrony between temperature and activity. Internal desynchrony has been suggested as the cause of impaired performance in a number of jet lag studies (see 22 for a review). However, this explanation seems unlikely because performance had returned to normal for both vigilance and discrimination, while activity had reached its new phase, but temperature was still resynchronizing, i.e., while the monkeys were still desynchronized. In addition, there was no sign of desynchronization when the second decline in performance occurred. Of course, the data do not enable us to exclude the possibility that internal desynchronization involving some unobserved variable(s) is responsible for the performance deficits.

The data that corresponded best with the performance data was the return to baseline performance patterns. There were task-dependent differences in the rate at which performance returned to its baseline pattern, with discrimination returning to the normal pattern earlier than vigilance. Monk and his colleagues (13) have reported similar differences in humans where tasks with high working memory loads resynchronized more rapidly than tasks with low working memory loads.

Further work is clearly needed to examine the role of task demands, such as memory load, and physiological factors on changes in performance across the day and following phase-shifts. However, the model reported here significantly advances our ability to study these problems in animals. The current model enables us to study physiological and behavioral processes in an integrated framework across the day and after manipulations that affect biological rhythms. Earlier animal work in the area typically examined

physiological and behavioral processes separately. Data from this study show that it is possible to study complex performance issues, such as task-dependent differences in the chronobiology of performance, in animals, and our current understanding suggests that understanding these differences may be crucial to understanding the role of the circadian system on performance in man and animals. Finally, the data reveal a considerable number of parallels between humans and rhesus monkeys in physiological and performance rhythms during both stable entrainment and following a phase-shift. These parallels lead us to conclude that most of the elements important to the chronobiology of performance can be studied in animals.

The availability of an animal model in this area has several advantages. First, the animal studies can be extended over longer time periods, allowing the emergence of previously unappreciated phenomena, such as the second decline in post-phase-shift performance reported here. Second, increased control of the environment and the increased ability to eliminate long-term trends due to learning and practice obviate methodological questions which have arisen in some human studies. Finally, the use of an animal model enables one to pursue questions that are not possible to pursue in humans. Examples of such areas might be in the use of new pharmacological treatments for the performance deficits of jet lag or in the investigation of the physiological processes involved in the arousal system that appears to modulate performance patterns across the day.

FOOTNOTES

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Requests for reprints should be addressed to W.N. Tapp, Primate Neurobehavioral Unit (127A), VA Medical Center, East Orange NJ 07019, USA.

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FIGURE LEGENDS

Figure 1: Schematic for task. On the average of every 2.4 min during the 8 hr work shift, a white cue light was illuminated. Pressing the middle lever within 10 sec initiated the discrimination trial (i.e., a red-green choice). Pressing the correct lever within 10 sec delivered a food pellet. A failure to press during either component of the task or the wrong choice during the discrimination component terminated the trial.

Figure 2: Average wave forms of temperature and activity from a representative monkey. Waveforms represent the averages of 20 consecutive days of data collected at 10 min intervals. Like this animal, the other monkeys also showed that activity rhythms (dashed line) peaked significantly before temperature rhythms (solid line).

Figure 3: Averaged vigilance (solid line) and discrimination (dashed line) performance from a representative monkey. For graphical purposes, the data have been transformed (reciprocal of latencies * 1000). The figure was prepared from data collected over 20 consecutive days during the 8 hr work shift. Like this animal, the other two monkeys also showed that discrimination performance peaked early in the work shift while vigilance performance peaked in the middle of the shift.

Figure 4: Rephasal of the 24 hr activity (dashed line) and temperature (solid lines) rhythms from a representative monkey. Like this animal, the other monkeys also showed that activity reached its new stable phase sooner than temperature.

Figure 5: Discrimination (top panel) and vigilance (bottom panel) performance from a representative monkey. The horizontal dashed line depicts the 99% confidence limits for baseline latencies. The dotted vertical line shows

the time of the 6 hr phase advance. Like this monkey, the other two monkeys also showed profound performance deficits following the phase-shift. The decrement in vigilance was greater and longer-lived than the decrement in discrimination. Note the second significant performance decrement beginning 10 days after the phase-shift. Although the time of this decrement varied slightly for the other two monkeys, it occurred invariably.

Figure 6: Rephasal of the 8 hr discrimination (X) and vigilance (O) performance after 6 hr phase advance (dotted vertical line). A template, consisting of hourly average latencies, was derived for each monkey based on 20 consecutive work shifts. Correlation coefficients indicate the correlation of each individual day's pattern with the template pattern. The horizontal dashed line is drawn at a correlation of 0.583 which is at the 0.05 level of significance with df = 7. As would be expected, individual days' data before the phase-shift correlate significantly with the template. Following phase-shift, correlations plummet. The pattern of discrimination performance returns to normal (i.e., shows a significant, positive correlation with the baseline template) significantly sooner than does vigilance performance. This difference parallels the faster recovery of performance efficiency in discrimination when compared to vigilance (see Figure 5). Note that at the time of the second decrement in performance, correlations with the template again drop. This means that not only is average performance affected in this period but also the pattern of performance too.

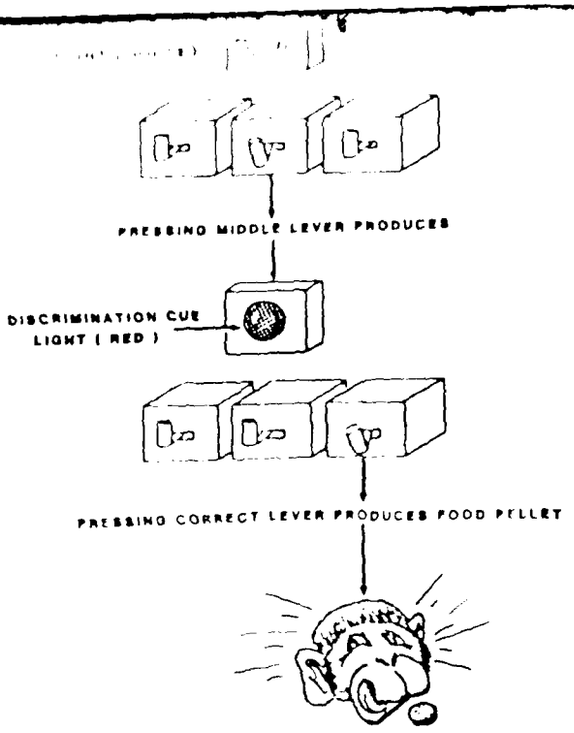


FIGURE 1

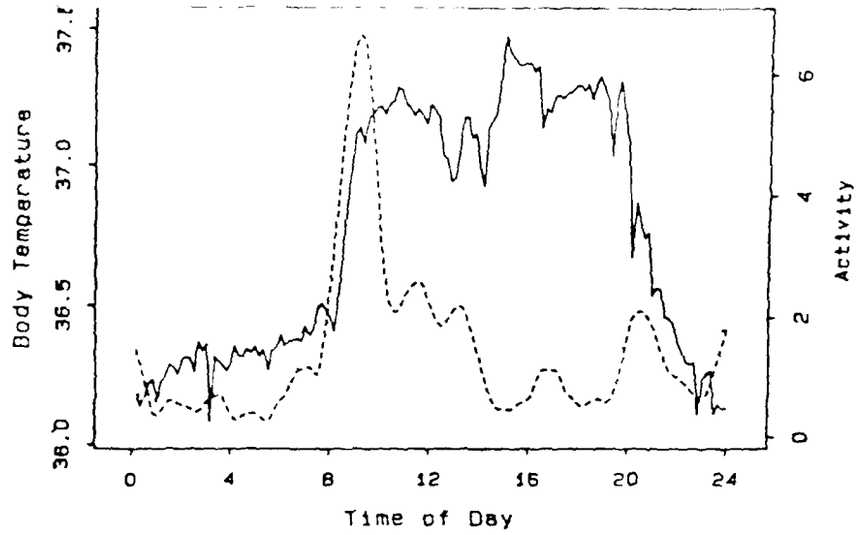


FIGURE 2

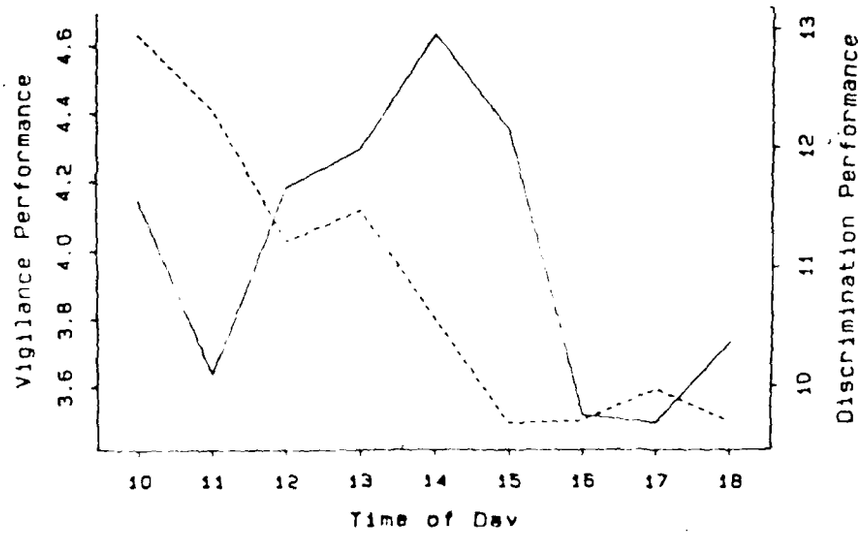


FIGURE 3

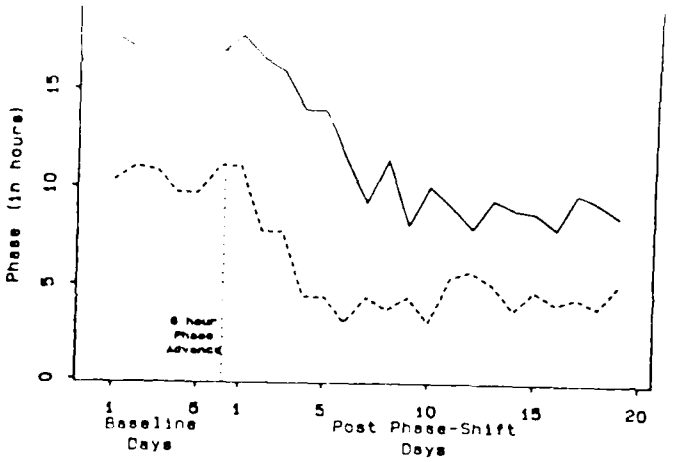
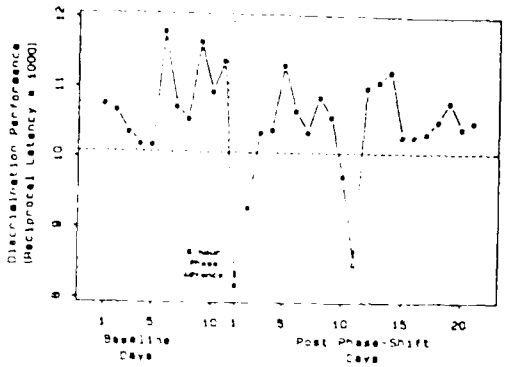


FIGURE 4



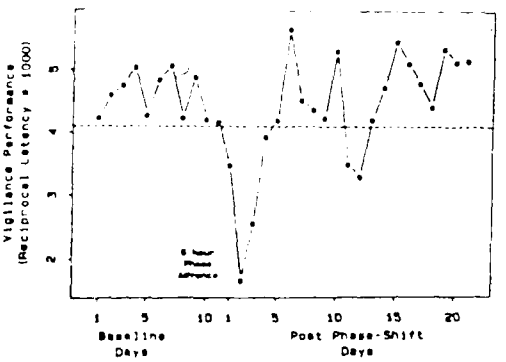


FIGURE 5

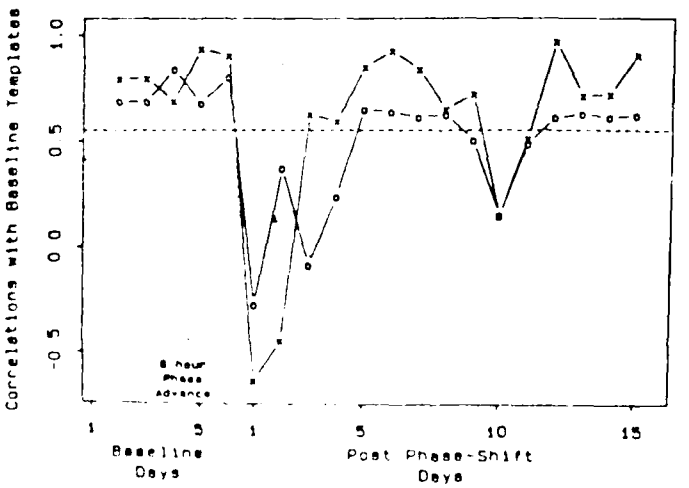


FIGURE 6

PUBLICATIONS SUPPORTED BY CONTRACT:

1. Natelson, B.H., Creighton, D., and Tapp, W.N. "Circadian factors in monkey performance: task differences and deficits after phase shifts." Fed. Proc. 44:1047, 1985.

2. Tapp, W.N., Natelson, B.H., and Creighton, D. "Circadian factors in monkey performance: Effects of task, phase shifts and constant light." Society for Neuroscience Abstracts, 11: 540, 1985.

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9. Tapp, W.N. and Natelson, B.H. Circadian rhythms and patterns of performance before and after simulated jet-lag. American Journal of Physiology, in revision.

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